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The BOTANICAL GAZETTE

FOUNDED BY JOHN M. COULTER

APRIL 1928

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THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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THE
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April 1928

LODGING IN OATS AND WHEAT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 377

F. A. WELTON

(WITH THIRTEEN FIGURES)

Introduction

Between erect and lodged grain no arbitrary line can sharply be drawn, since the one condition merges gradually into the other. In this paper the term erect is applied to grain which at harvest time stands upright, or nearly so. By lodged grain is meant that which has partly or completely lain over on the ground and which may have become more or less tangled. In some lodged grain the culms simply lie over on the ground without any perceptible bend at the base of culm; in other cases there is an abrupt bend in the culm at one of the lower nodes, in which case a field presents an appearance like that shown in fig. 1. Occasionally the bend occurs at one of the upper nodes, and then a situation develops like that presented in fig. 2.

Lodging may affect very materially both the quantity and quality of the grain, the degree of loss depending largely on the completeness of the falling, the state of development at which it occurs, and the subsequent weather conditions. Frequently the loss due to imperfect development of kernels may range from five to ten or more bushels per acre.

Historical

The work heretofore done on lodging may, in a broad way, be grouped into three classes: observational, anatomical, and chemical.

OBSERVATIONAL.—THÄER (32), an English physician and agriculturist, was among the earliest to take note of lodging in wheat, and he attributed it to rich ground, abundant manuring, poor plowing, thick sowing, and sometimes to a diseased condition. KUHN (18), MAYER (21), VON SEELHORST (37), HOUTMAN (8), HALL (6), and many others have observed that lodging is often associated with the presence of excessive nitrates or with rich ground in general. The various constituents contained in fertilizers have also been mentioned. THORNE and HICKMAN (33), for example, observed more

FIG. 1.—Field of lodged rye, showing most of the culms bent over abruptly near one of the lower nodes.

lodging in wheat from the use of a mixture of phosphate and nitrate of soda than from the use of either alone. MURRAY (23) observed less lodging in rye where the fertilizers contained some potash. HARCOURT (7) attributed the difference observed in a field of oats to the presence of more lime and less nitrogen in the part that remained erect. In an experiment with oats in Scotland (2), a top dressing of nitrate of soda increased lodging, and common salt appeared to be more efficient than did potash as a preventative.

ANATOMICAL.—LIENAU (20), VAGELER (36), ALBRECHT (1), MOLDENHAUER (22), GARBER and OLSON (5), PURVIS (27), and others have studied lodging in relation to the anatomical structure of the plants. Most of these workers failed to find any relationship be-

tween the anatomical structure and lodging. GARBER and OLSON, however, concluded that thinness of cell walls seemed to be associated with lodging.

CHEMICAL.—Weakness of straw has been attributed to lack of various elements, particularly silicon and potassium. That lack of silicon is responsible for lodging seems first to have been suggested by DAVY (3), who, as early as 1789, discovered that in the epidermis of rattan there is a sufficient quantity of flint to produce light when

FIG. 2.—*Unusual type of lodging in wheat, showing many of the culms bent over* abruptly near first or second node below the head.

struck by steel. As a result of this and other observations, he conducted certain experiments from which he concluded that siliceous *earth is common in the epidermis of hollow plants, and that it* serves as a support.

LIEBIG (19) also believed that weakness of culms was due to lack *of an abundance of silicon. He ascribed the strength of all stems of* the grass family to silicate of potash. The importance of silicon was emphasized also by KUHN (18), SWIECICKI (31), and KOHL (15). As *a result of the work of* SACHS (30), KNOP (13), JODIN (9), PIERRE (26), MAYER (21), and others, the importance of silicon began to be questioned. As late as 1916, however, DOUGLAS (4) gave lack of

silicic acid as one of many causes for lodging in sugar cane. Perhaps its presence renders the cell walls more resistant to attacks of insect pests and fungous diseases, for JULIEN and DUPONT (10) reported a case in which lodging of wheat was thought to be due to an infestation of two fungous diseases.

Straw weakness has also been attributed to lack of lignification in the supporting tissues in the culms. SACHS (30) observed that such a situation usually develops where the stand of plants is too thick, the culms therefore shading one another unduly and thus taking on the diseased condition of etiolation. Lack of sufficient light was considered as an important factor by KOCH (14), KRAUS (16), RIVERA (29), WIESSMAN (39), PERCIVAL (25), PALLADIN (24), and others.

SUMMARY.—From the foregoing review it is clear that lodging has long been associated with hypernutrition, particularly of a nitrogenous nature. It is also apparent that many have considered it as related to reduced light, but no suggestion has been given as to how these two factors may interact with each other to bring about such a condition. The importance of silica from the standpoint of straw strength seems to have fallen into disrepute, by reason of the fact that plants have been found to make apparently normal growth even to maturity in nutrient solutions free from silicic acid. In the development of the silicic acid theory, the fact seems to have been overlooked that much of the silica found in the grasses is contained in the leaves rather than in the culms.

Object

In a broad way lodging may be said to result from two sets of causes: (1) the interaction of those environmental factors which make for the development of weak stems; and (2) external forces which exert no influence on the structure of the stems but which cause lodging through mechanical impact, such as violent wind, rain, or hail storms. For obvious reasons this discussion is not concerned with the latter set of causes. The purpose of this paper has been to ascertain the environmental conditions responsible for the development of weak stems in oats and wheat, and to trace the chain of se-

quence from the non-lodged to the lodged condition through the internal responses produced by the interaction of the various factors involved.

Material and methods

For this study there were used mainly pure line strains of oats and wheat. Some of the material was grown in pots in a greenhouse, and some (the major part) under field conditions.

For a study of the physical characteristics of lodged and non-lodged grain, samples of fully developed culms were gathered from definite areas in general fields, shortly before harvest. From these the stand of plants was determined, also the proportion of straw and grain. Other culms were gathered at the heading stage while the plants were green, and on these the diameter and breaking strength of the culms were measured.

For the chemical studies, 100 gm. samples of culms were gathered at heading time and preserved in alcohol. The samples of so-called lodged culms were composed in part of culms partially lodged and in part of erect culms selected from the border of lodged areas. Completely prostrate culms were avoided because it was thought that the bend in such stems might interfere with the normal translocation of nutrients and hence alter the composition. The samples of erect or normal grain were selected in regions where all the surrounding culms were standing erect. All the samples consisted of the lower third of the culms from which the leaves had been removed. The stems were cut into pieces one-half to one inch in length, placed in wide-mouthed, ground-glass stoppered bottles, and then covered with alcohol; the strength of the final solution, including the moisture contained in the stems, being about 70 per cent. After heating about one hour at approximately 78° C., the material was set aside and allowed to stand for at least two months. On these samples analyses were made for moisture and dry matter, nitrate and total nitrogen, and various carbohydrate compounds including cellulose, lignin, and sugars of different kinds.

Microchemical tests were made on fresh material for nitrates, free-reducing substances, cellulose, lignin, and to a less extent for some of the mineral substances such as potassium and calcium.

For the anatomical study a few typical culms of green stems were selected at heading time and preserved in a formalin-alcohol solution. Freehand sections were made and stained in safranin and light green.

Results

FIELD SURVEY

Samples of lodged and non-lodged oats were gathered from the field at harvest time in 1920, 1921, 1922, 1923, and 1924. In each year ten samples of each kind were selected. Each sample represented the harvest from an area one yard square. In 1920 the

TABLE I

PERCENTAGE OF STRAW IN ERECT AND LODGED OATS

	1920 Ohio 6222		1921 Ohio 201		1922 Ohio 201		1923 Ohio 201		1924 Ohio 201	
	Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged
	62.1	77.3	64.8	75.2	92.6	87.7	60.5	64.5	61.1	67.2
	59.0	79.6	62.9	76.4	94.4	86.5	57.0	73.8	60.6	68.0
	61.8	81.8	64.1	74.1	93.7	82.2	60.0	62.4	58.4	64.0
	63.6	85.5	65.0	74.4	90.6	90.3	59.9	68.9	59.9	65.9
	60.2	79.6	65.5	73.5	92.1	91.6	62.8	67.1	62.7	64.3
	59.1	93.2	69.8	75.1	93.4	86.9	59.3	73.1	60.6	64.3
	61.9	90.8	68.8	78.1	84.9	92.5	65.4	68.6	58.1	67.6
	60.6	95.9	68.0	75.9	70.7	76.4	60.8	71.1	56.7	62.4
	59.8	93.3	66.5	74.3	92.2	92.8	64.1	65.3	53.0	63.7
	61.4	94.4	66.4	77.6	92.3	91.8	58.9	65.4	58.4	70.2
Average....	60.9	87.1	66.2	75.4	89.7	87.7	60.5	67.7	59.0	65.8

samples consisted of a pure line strain of the Improved American variety, Ohio 6222; in the other years they consisted of the Ohio 201, a selection from the Siberian variety. After weighing, each sample was threshed and the proportion of grain and straw calculated. The percentage of straw found in each individual sample of erect and lodged grain and the average of the ten samples of each kind for each year are given in table I. From this table it may be noted that the average percentage of straw was higher in the lodged than in the erect grain in every year except 1922. In that year a violent wind and rain storm caused most of the field to lodge, and this rendered difficult an accurate separation of the erect from what would probably have lodged under normal conditions.

Square yard areas of erect and lodged wheat were gathered in a similar way at harvest time in the years 1919, 1920, 1921, 1923, and 1924. In 1919 the samples were gathered from two varieties, Gladden and Fulhio; in 1920 from the Fultz and Fulhio; and in each of the other years from one variety, the Fulhio. With the exception of the Fultz these varieties are pure lines. After weighing and threshing, the percentage of straw was calculated; and the percentage of straw for each sample and the average percentage for each class for each year are given in table II. From this table it may be noted that the

TABLE II

PERCENTAGE OF STRAW IN ERECT AND LODGED WHEAT

1919				1920				1921		1923		1924	
GLADDEN		FULHIO		FULTZ		FULHIO		FULHIO		FULHIO		FULHIO	
Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.
71.4	80.7	69.6	73.4	52.4	76.8	61.2	73.5	69.9	79.2	66.9	73.7	56.0	67.2
68.0	85.3	72.5	74.2	70.5	75.4	63.0	71.8	78.8	75.5	63.1	76.0	63.3	72.9
74.6	79.3	70.3	75.3	68.7	75.2	65.3	72.2	76.1	72.4	63.1	70.3	62.9	74.0
71.5	78.8	72.4	74.1	69.8	75.5	60.6	70.5	79.2	81.1	61.9	71.7	69.1	65.3
71.2	83.3	71.4	74.8	70.2	74.1	61.4	72.5	70.4	72.5	61.9	70.1	70.1	75.2
70.9	81.5	72.7	79.2	69.3	73.7	62.2	74.1	69.1	71.1	85.9	73.3	68.1	66.9
71.7	86.9	69.5	74.7	71.2	81.1	64.5	71.8	69.1	70.3	67.8	71.9	60.8	73.8
70.4	73.5	68.2	71.6	69.4	80.1	67.9	71.3	68.9	73.9	69.6	74.2	67.9	71.8
71.0	74.0	69.3	73.5	70.2	78.2	65.7	71.7	68.9	69.7			76.0	72.7
71.8	87.2	68.6	74.9	69.6	80.2	66.1	70.4	70.5	71.8			65.6	66.1
Average.....71.2	81.0	70.4	74.5	68.1	77.0	63.7	71.9	72.1	73.7	67.0	72.6	66.0	70.6

average percentage of straw was higher in the lodged than in the erect grain in every year and in all the varieties. A part of the higher percentage of straw is due probably to a tendency toward the development of slightly longer heads in lodged than in erect grain. Measurement of the length of 1000 heads each of lodged and erect oats gave an average length of 275.8 and 255.1 mm. respectively. Although longer, the lodged heads were lighter, for the average weight per head was 1700 and 1890 mg. for the lodged and erect heads respectively. The lighter weight is due to the development of more shriveled kernels in lodged plants, and this is reflected in the weight per bushel of the threshed grain. In a field of oats, a part of which

lodged about heading time, the weight of seeds per bushel grown on lodged and erect plants was 22 and 33 lb. respectively.

In wheat also the lodged heads are longer but lighter. The average length of 1000 heads each of lodged and erect wheat was found to be 75.0 and 72.1 mm. respectively. The average weight per head of the same two lots of lodged and erect wheat was 830 and 900 mg. respectively.

The higher percentage of straw in lodged plants is due chiefly to the fact that in such the number and length of culms are greater

TABLE III

NUMBER OF CULMS PER SQUARE YARD IN ERECT AND LODGED OATS

	1920 Ohio 6222		1921 Ohio 201		1922 Ohio 201		1923 Ohio 201		1924 Ohio 201	
	Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged	Erect	Lodged
	266	344	214	221	163	183	216	222	226	268
	285	366	129	244	202	185	211	201	188	287
	287	322	178	218	208	240	175	220	169	243
	274	333	158	222	196	238	221	233	180	262
	263	323	157	215	224	232	213	276	175	233
	275	343	156	313	167	218	205	230	162	252
	258	316	226	179	194	228	202	234	160	286
	272	318	181	197	175	199	246	214	143	270
	315	321	187	246	204	227	228	268	137	289
	273	331	204	248	200	231	222	240	179	277
Average....	277	329	179	230	193	218	213	233	172	267

than in erect ones. That the number of culms is greater is shown by counts made on the same samples of oats and wheat for which the percentages of straw were given, as indicated in tables I and II. The counts were made before the grain was threshed, and the results obtained are recorded for the oats in table III and for the wheat in table IV. From table III it may be noted that the average number of culms was greater in the lodged than in the erect oats in each of the five years, the difference ranging from 16 to 74 per cent.

The denser stand of lodged grain is due probably to a relatively higher moisture and nitrate content of the soil, for almost invariably lodged grain is found on the lower and more fertile land, in depressions, ravines, etc., rather than on knolls, hills, or upland in general.

That relatively high moisture content of the soil tends to induce tillering was shown by a pot test conducted in the greenhouse during

the winter of 1919–1920. In this test two lots each of 42 one-gallon jars were filled with an equal quantity of rich compost, to which subsequently had been added and thoroughly mixed with it a liberal application of both lime and acid phosphate. In each jar four seedlings of pure line wheat, Ohio 13384, were planted January 9, 1919. The two lots of jars were treated alike in all respects except in the amount of water given; one lot being kept at optimum minus, the other at optimum plus. The average number of culms produced in

TABLE IV

NUMBER OF CULMS PER SQUARE YARD IN ERECT AND LODGED WHEAT

	1919				1920				1921		1923		1924	
	GLADDEN		FULHIO		FULTZ		FULHIO		FULHIO		FULHIO		FULHIO	
	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.	Erect	Ldg.
	462	561	460	577	407	624	302	609	436	610	555	597	483	799
	473	695	390	540	406	579	282	519	445	582	558	513	595	763
	591	678	429	535	406	576	367	711	460	605	498	714	547	666
	480	560	421	667	404	592	274	473	493	617	565	493	564	545
	437	653	406	537	389	613	211	645	478	500	608	590	559	616
	476	645	380	582	394	535	282	667	454	539	463	510	444	752
	480	688	577	628	380	658	424	425	471	548	544	675	536	474
	429	554	420	477	446	631	401	490	440	484	410	779	466	669
	405	513	441	615	391	685	348	497	461	472			539	625
	393	651	482	540	460	644	370	504	447	485			492	738
Average.....	462	619	440	569	408	614	318	554	458	544	525	609	523	665

the optimum minus and optimum plus jars was 7.4 and 8.5 respectively at the jointing stage. Many of the smaller culms died and withered away as the plants developed.

The addition of nitrates also tends to increase the amount of tillering. In a pot tested with wheat conducted in the greenhouse during the winter of 1920–1921, nitrate of soda was applied at different dates, the primary object being to note the effect of such applications on the quality of the grain. Incidentally the amount of tillering was noted. The wheat to which the nitrate of soda was applied averaged 3.0 culms per plant, while the untreated averaged 2.2 culms. A similar test made in the winter of 1921–1922 gave 2.7 culms and 2.0 culms per plant for the treated and untreated wheat respectively.

As a rule the straw of lodged grain is relatively soft and pliable as compared with that of erect grain, thus suggesting a variation in chemical composition. Accordingly samples grown under various conditions favorable for non-lodging and lodging were selected for chemical analysis.

CHEMICAL COMPOSITION

HIGH AND LOW GROUND.—Two samples each of oats and wheat grown in general fields were selected in 1924. One sample of each was selected from among erect grain; and one of each from among

TABLE V

COMPOSITION OF OAT AND WHEAT CULMS GROWN IN A GENERAL FIELD ON LOW AND HIGH GROUND IN 1924

MATERIAL	PERCENTAGE			
	OATS		WHEAT	
	Low ground (rich)	High ground (poor)	Low ground (rich)	High ground (poor)
Moisture	76.400	68.880	78.450	69.380
Dry matter	23.600	31.120	21.550	30.620
Nitrate nitrogen	0.003	0.011	0.001	0.002
Total nitrogen	0.072	0.068	0.082	0.053
Free reducing sugars	1.742	2.802	2.439	2.091
Disaccharides	0.174	4.051	1.684	9.932
Polysaccharides	3.854	5.221	4.201	5.484
Cellulose	3.725	3.259	3.379	3.021
Lignin	10.171	10.884	6.866	6.471
Total carbohydrates	19.666	26.217	18.569	26.999

that which was badly lodged. The lodged areas were located on relatively low ground. Naturally, such ground is more fertile and contains more moisture than the higher ground, for it receives the seepage from the latter. The distance was a matter of a few yards only in most cases. The results of the analysis are recorded in table V. From the table it may be noted that in both oats and wheat the culms of the plants grown on the low ground contained more moisture, slightly more total nitrogen, less dry matter, and less total carbohydrates than did those grown on the relatively high ground.

SAND-SOIL-MANURE TEST.—In order to study the effect of more extreme conditions of fertility on lodging, both oats and wheat were

grown in soils artificially prepared and representing wide variations, particularly as regards nitrates. The poor soil consisted of one part by measure of good silt loam mixed with three parts common creek sand. The sand had previously been passed through an ordinary sand screen in order to remove large stones and other foreign materials. The rich soil was made by mixing with the silt loam some well rotted manure in the proportion of one part by measure of soil and three parts of manure. The two classes of artificial soil were prepared to a depth of 7 inches, ordinary plow depth, and each embraced an area of one square yard. They were arranged one on

TABLE VI

EFFECT OF FERTILITY ON COMPOSITION OF OAT AND WHEAT CULMS, 1922

MATERIAL	PERCENTAGE					
	OATS			WHEAT		
	Sand ¾ Soil ¼	Soil	Soil ¼ Manure ¾	Sand ¾ Soil ¼	Soil	Soil ¼ Manure ¾
Moisture	74.039	72.555	80.670	67.711	69.941	74.966
Dry matter	25.961	27.445	19.330	32.289	30.059	25.034
Nitrate nitrogen	0.002	0.004	0.018	0.000	0.001	0.021
Total nitrogen	0.076	0.062	0.080	0.154	0.138	0.197
Free reducing sugars	2.803	2.090	1.178	1.443	1.443	0.661
Disaccharides	0.000	0.000	0.210	3.283	2.375	1.468
Polysaccharides	4.587	4.925	2.994	5.599	5.314	4.163
Cellulose	4.443	4.957	3.249	4.468	4.647	4.277
Lignin	8.776	8.982	5.894	10.460	10.408	7.981
Total carbohydrates	20.609	20.954	13.525	25.253	24.187	18.550

either side of an equal area of the natural soil, the Wooster silt loam, and were separated by one-inch boards. The three classes are designated in this paper as sand-soil-manure. Soils of these three grades were prepared for the growing of both oats and wheat. Crops of each were harvested and sampled for analysis in 1922, 1923, and 1924. The results of the analysis are given in tables VI, VII, and VIII.

From the tables it may be seen that in both oats and wheat and in all three years the plants grown in the manure, as contrasted with those grown in the sand or soil, were in general high in moisture and consequently low in dry matter and in total carbohydrates, including polysaccharides, cellulose, and lignin. They were also higher in

nitrate nitrogen and total nitrogen. Nitrate determinations made on the three classes of soil in 1923 showed 2.4, 3.9, and 135 parts per million in sand-soil-manure respectively.

TABLE VII

EFFECT OF FERTILITY ON COMPOSITION OF OAT AND WHEAT CULMS, 1923

MATERIAL	PERCENTAGE					
	OATS			WHEAT		
	Sand ¾ Soil ¼	Soil	Soil ½ Manure ½	Sand ¾ Soil ¼	Soil	Soil ½ Manure ½
Moisture	73.780	72.920	77.340	71.760	73.820	79.230
Dry matter	26.220	27.080	22.660	28.240	26.180	20.770
Nitrate nitrogen	0.021	0.017	0.058	0.003	0.006	0.066
Total nitrogen	0.180	0.181	0.357	0.176	0.173	0.278
Free reducing sugars	1.918	2.235	1.924	2.016	2.396	0.783
Disaccharides	0.596	1.514	2.001	5.359	3.152	0.954
Polysaccharides	6.064	5.733	4.115	5.653	5.332	3.960
Cellulose	3.315	3.342	2.550	3.111	3.085	2.977
Lignin	7.332	7.667	5.850	6.771	7.078	6.002
Total carbohydrates	19.225	20.491	16.440	22.910	21.043	14.676

TABLE VIII

EFFECT OF FERTILITY ON COMPOSITION OF OAT AND WHEAT CULMS, 1924

MATERIAL	PERCENTAGE					
	OATS			WHEAT		
	Sand ¾ Soil ¼	Soil	Soil ½ Manure ½	Sand ¾ Soil ¼	Soil	Soil ½ Manure ½
Moisture	76.060	77.080	80.230	59.000	69.800	79.380
Dry matter	23.940	22.920	19.770	41.000	30.200	20.620
Nitrate nitrogen	0.001	0.005	0.123	0.001	0.003	0.052
Total nitrogen	0.048	0.044	0.215	0.095	0.062	0.116
Free reducing sugars	1.670	1.771	0.537	1.916	2.129	1.045
Disaccharides	2.918	2.004	1.670	9.584	7.860	1.103
Polysaccharides	4.498	4.273	3.419	9.144	5.452	3.867
Cellulose	3.133	3.270	2.713	4.748	3.030	3.235
Lignin	9.540	8.919	6.535	11.896	7.890	7.180
Total carbohydrates	21.759	20.237	14.874	37.288	26.361	16.430

In general the stems of the plants grown in the soil, and especially those grown in the sand, were small and tough, while those grown in the manure were much darker in color. In each year the oats and

wheat grown in the manure lodged, or showed a strong inclination to lodge, while those grown in the soil and sand stood perfectly erect.

On the manure more and longer culms developed, thus resulting in a greater percentage of straw. The heavier growth produced more shade, and the difference in the intensity of this at the base of the culms, as measured by RIDGEWAY'S (**28**) chemical photometer, is indicated in table IX.

The percentages refer to the amount of oxalic acid decomposed under the influence of the light that struck the lower part of the

TABLE IX

INTENSITY OF LIGHT AMONG OAT AND WHEAT PLANTS GROWN ON RICH AND POOR SOIL

TREATMENT	PERCENTAGE	
	Oats	Wheat
Sand	100	100
Soil	20	12
Manure	0	4

culms growing in the different kinds of soil. The amount decomposed among the plants growing in the sand was recorded as 100 per cent. The proportionate amount decomposed among the plants grown in the soil and manure was computed and recorded in percentage also. The percentages, therefore, represent not the absolute but the relative amount of light surrounding the lower part of the culms.

The low intensity of light among the plants grown in the manure may have been responsible in part for the relatively low carbohydrate content, because such conditions are not conducive to maximum carbon assimilation.

SHADING.—In order to discover the effect, if any, of reduced light on the composition of the stems, small areas of oats and wheat growing in general fields were covered with cheesecloth. Frames about 12 feet square and 5 feet high, as shown in fig. 3, were constructed and covered with a single layer of cheesecloth, ample provision being made for the free circulation of air. The frames were placed in position before the oats and wheat came into head, and were allowed to remain there until harvest. This was done in four

seasons, 1921, 1922, 1923, and 1924. In 1923 and 1924 samples of oats and wheat culms grown inside and outside the cheesecloth frames were taken at heading time. The analysis of the 1923 samples is given in table X, and of the 1924 samples in table XI.

From these tables it may be seen that a marked reduction in dry matter and total carbohydrates was found in the shaded oats and wheat in both years. The reduction occurred principally in the disac-

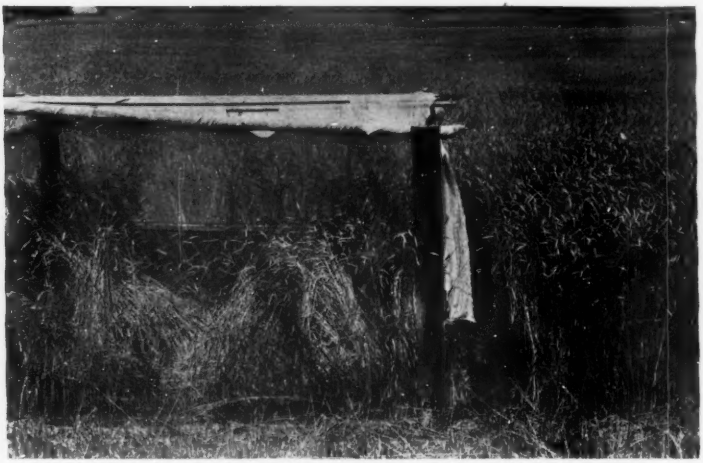


FIG. 3.—Shaded and unshaded wheat

charides, polysaccharides, and lignin, it being most marked in the disaccharides.

The oats and wheat covered with cheesecloth grew slightly taller than the uncovered. In two of the four seasons there was some lodging in the general fields but none in the vicinity of the cages. In all four years, however, the covered grain lodged. Fig. 3 shows the lodging of wheat when shaded in this manner.

Under field conditions reduced light around the lower part of the culms may result from such cultural practices as thick seeding or the use of small seeds. In 1924 samples were gathered from oats seeded at the rates of 4, 14, and 24 pecks per acre, and from wheat sown at the rates of 3 and 10 pecks per acre. The analytical results, given in

table XII, do not show any material difference in the percentage composition of dry matter and total carbohydrates due to rate of seeding. However, on account of the reduced size in diameter of the

TABLE X

EFFECT OF SHADE ON COMPOSITION OF OAT AND WHEAT CULMS, 1923

MATERIAL	PERCENTAGE			
	OATS		WHEAT	
	Unshaded	Shaded	Unshaded	Shaded
Moisture	79.810	84.090	61.500	65.600
Dry matter	20.190	15.910	38.500	34.400
Nitrate nitrogen	0.034	0.027	0.020	0.002
Total nitrogen	0.159	0.172	0.118	0.129
Free reducing sugars	2.408	1.279	1.786	2.799
Disaccharides	0.819	0.508	8.968	0.840
Polysaccharides	3.596	2.621	7.078	6.353
Cellulose	2.606	2.156	4.275	4.850
Lignin	6.125	4.983	11.418	10.806
Total carbohydrates	15.554	11.547	33.525	25.648

TABLE XI

EFFECT OF SHADE ON COMPOSITION OF OAT AND WHEAT CULMS, 1924

MATERIAL	PERCENTAGE			
	OATS		WHEAT	
	Unshaded	Shaded	Unshaded	Shaded
Moisture	77.230	84.670	75.830	78.860
Dry matter	22.770	15.330	24.170	21.140
Nitrate nitrogen	0.004	0.002	0.001	0.002
Total nitrogen	0.103	0.059	0.060	0.067
Free reducing sugars	1.830	0.494	2.497	2.280
Disaccharides	0.378	0.000	7.086	1.902
Polysaccharides	4.283	2.969	4.551	3.989
Cellulose	3.412	2.899	2.932	2.980
Lignin	9.565	6.235	6.758	6.586
Total carbohydrates	19.468	12.597	23.824	17.737

culms, there was a material reduction in the quantity of all the constituents per given length of culm, as shown in table XIII.

Similar results were obtained from the use of large and small oat seeds. In a field plot test with oats, seeds of different grades were

used. The difference in size of kernels is such as can be obtained by passing the seeds repeatedly through a good fanning mill. In 1924, after the oats were cleaned, the calculated number of seeds per

TABLE XII

EFFECT OF RATE OF SEEDING ON COMPOSITION OF OAT AND WHEAT CULMS, 1924

MATERIAL	PERCENTAGE				
	OATS IN PECKS PER ACRE			WHEAT IN PECKS PER ACRE	
	4	14	24	3	10
Moisture	80.360	79.500	80.950	73.460	72.770
Dry matter	19.640	20.500	19.050	26.540	27.230
Nitrate nitrogen	0.049	0.003	0.005	0.004	0.003
Total nitrogen	0.069	0.062	0.067	0.126	0.104
Free reducing sugars	1.829	1.670	1.147	8.480	6.185
Disaccharides	0.261	0.653	0.944	0.073	0.000
Polysaccharides	3.177	3.353	3.300	5.516	5.114
Cellulose	2.925	3.497	3.181	3.104	3.974
Lignin	8.636	8.686	7.908	8.434	9.074
Total carbohydrates	16.828	17.859	16.480	25.607	24.347

TABLE XIII

EFFECT OF RATE OF SEEDING ON COMPOSITION PER UNIT LENGTH OF CULM

MATERIAL	CONSTITUENTS PER CENTIMETER OF CULM (MG.)				
	OATS IN PECKS PER ACRE			WHEAT IN PECKS PER ACRE	
	4	14	24	3	10
Moisture	99.209	67.948	37.476	56.725	30.601
Dry matter	24.247	17.521	8.819	20.494	11.451
Total nitrogen	0.085	0.053	0.031	0.097	0.044
Free reducing sugars	2.258	1.427	0.531	6.548	2.601
Disaccharides	0.322	0.558	0.437	0.056	0.000
Polysaccharides	3.922	2.866	1.528	4.259	2.151
Cellulose	3.611	2.989	1.473	2.397	1.671
Lignin	10.661	7.424	3.661	6.513	3.816
Total carbohydrates	20.775	15.264	7.629	19.773	10.238
No. culms per 100 gm	18	30	60	35	58
Av. length of culms cm	45	39	36	37	41
Total length of culms	810	1170	2160	1295	2378

bushel was 779,520 for the large and 1,835,520 for the small. The two classes of seeds were sown at three different rates: 4, 14, and 24 pecks per acre. The composition of the culms, given in table XIV,

does not show any material difference in the percentage of dry matter and total carbohydrates. The culms produced from the small seeds,

TABLE XIV

EFFECT OF SIZE OF SEED ON COMPOSITION OF OAT CULMS, 1924; LARGE VERSUS SMALL SEED OATS

MATERIAL	PERCENTAGE					
	4-PECK RATE		14-PECK RATE		24-PECK RATE	
	Large	Small	Large	Small	Large	Small
Moisture	81.760	79.560	78.260	77.450	78.740	78.450
Dry matter	18.240	20.440	21.740	22.550	21.260	21.550
Nitrate nitrogen	0.002	0.003	0.002	0.002	0.001	0.002
Total nitrogen	0.068	0.062	0.067	0.056	0.073	0.056
Free reducing sugars	1.510	1.786	1.481	1.554	1.525	1.771
Disaccharides	0.639	0.886	1.829	1.525	1.670	1.074
Polysaccharides	3.114	3.382	3.771	3.924	4.019	3.584
Cellulose	2.920	2.965	3.158	3.160	3.018	3.382
Lignin	7.796	8.554	8.642	8.648	8.512	9.340
Total carbohydrates	15.979	17.573	18.881	18.811	18.744	19.151

TABLE XV

EFFECT OF SIZE OF SEED ON COMPOSITION PER UNIT LENGTH OF OAT CULM

MATERIAL	CONSTITUENTS PER CENTIMETER OF CULM IN MG.					
	4-PECK RATE		14-PECK RATE		24-PECK RATE	
	Large	Small	Large	Small	Large	Small
Moisture	100.073	61.818	63.110	31.716	38.001	27.938
Dry matter	22.325	15.882	17.532	9.234	10.260	7.675
Total nitrogen	0.082	0.048	0.054	0.023	0.035	0.020
Free reducing sugars	1.848	1.388	1.194	0.636	0.736	0.631
Disaccharides	0.782	0.688	1.475	0.624	0.806	0.382
Polysaccharides	3.812	2.628	3.041	1.607	1.939	1.276
Cellulose	3.574	2.304	2.547	1.294	1.457	1.204
Lignin	9.542	6.646	6.696	3.541	4.108	3.330
Total carbohydrates	19.558	13.654	15.226	7.703	9.046	6.820
No. culms per 100 gm.	19	33	31	74	56	78
Av. length of culms cm.	43	39	40	33	37	36
Total length of culms	817	1287	1240	2442	2072	2808

however, were materially smaller in diameter, and on account of this fact the constituents per given length of culm, as shown in table XV, were significantly reduced.

DIAMETER AND BREAKING STRENGTH OF CULMS.—Either thick seeding or the use of small seeds gives an increase in the stand of plants, and this results in a decrease in the diameter and hence in the breaking strength of culms. The average diameter and breaking strength of oats sown at the rates of 4 and 14 pecks and of wheat sown at the rates of 3 and 10 pecks per acre, and of oats grown from large and small seed are shown in table XVI. The weight of 100 sections, each taken from a separate culm, is also shown for each of these classes of seed.

From table XVI it is evident that a thickening of the stand affected adversely in all cases the strength of the culms. It decreased

TABLE XVI

EFFECT OF THICK STAND ON DEVELOPMENT OF CULMS

MATERIAL	OATS			WHEAT		
	Diameter (mm.)	Breaking strength (gm.)	Weight of 100 sections 8 cm. long (gm.)	Diameter (mm.)	Breaking strength (gm.)	Weight of 100 sections 8 cm. long (gm.)
Thin seeding	5.24	1329	134.5	3.39	573	91.0
Thick seeding	4.09	712	78.2	2.83	370	63.1
Large seed	4.61	1115	100.0	.....	.....	.....
Small seed	3.60	690	78.0	.....	.....	.....

the diameter and breaking strength and reduced the density, as indicated by the weight of a group of sections of definite number and length.

The culms were gathered soon after the grain had reached the full heading stage, and the weights and measurements were made on the fresh material. In the oats the diameter and breaking strength were determined at the fourth node below the head, the diameter being measured just below and the breaking strength on the node. In the wheat the determinations were made in the same way, but at the third node below the head.

In an annual rate-of-seeding field test with oats it has often been observed that the degree of lodging is correlated with the rate of seeding; the heavier the rate the greater the amount of lodging. In this test the rates of seeding vary from one another by one peck, and

they extend from 4 to 14 pecks inclusive. In lodging years the falling down usually begins at the 5- or 6-peck rate, and increases as the rate of seeding increases. Figs. 4 and 5 show the erect culms from seeding oats at 4 pecks per acre and the lodging produced when 14 pecks per acre are used.

FIGS. 4, 5.—Oats: fig. 4, four pecks per acre; fig. 5, fourteen pecks per acre

TEMPERATURE.—In order to determine whether there is any relationship between temperature and lodging, an area each of oats and wheat approximately 6 by 6 feet was inclosed with window glass. Of course the glass changed the quality of light, and in order to overcome this source of error a second area of both oats and wheat,

each approximately 12 by 12 feet, was covered with window glass, the sides being left open so as not to affect appreciably the temperature. The covering was raised from time to time as the oats and wheat increased in height. It was thought that this arrangement would result in light of the same quality in both areas, and that any difference in composition of the plants grown under the two conditions might therefore be attributed, in large measure at least, to the higher temperature in the inclosure. Of course there was some difference in humidity, but this was not great. The rate of evaporation, as shown by atmometers, was somewhat greater inside than outside the inclosures. No artificial heat was applied, and hence the amount of increase within was dependent entirely on the outside temperature. On hot sunshiny days the rise was higher than on cool cloudy days. On warm days the maximum increase ranged from 8° to 10° F. The temperature under the glass cover, even on the warmest days, rarely exceeded that outside by more than one or two degrees.

Small areas of oats were covered with these two kinds of frames June 26, 1924, when the plants were about 18 inches tall and growing vigorously. In the inclosure the oats lodged badly on July 10, at which time they were well in head. At the same time there was slight indication of lodging under the glass cover, but the oats did not go down there until considerably later, and never as badly as in the inclosure. On July 22 a sample was taken from the inclosure, one from under the glass cover, and one from the open. The oats in the inclosure were preceptibly taller than were those under the glass cover, and those under the cover were somewhat taller than were those growing in the open.

Areas of wheat were covered with similar frames of glass April 23, 1924, at which time the young wheat was approximately 4 inches tall. The wheat in the glass inclosure headed out about two days in advance of that under the glass cover, and the latter preceded the general field by approximately one day. The wheat in the inclosure showed first heads June 7. It lodged badly June 14, at which time the wheat under the glass cover also showed a slight tendency to lodge. Later the lodging of the wheat under the glass cover became more pronounced, but it never equaled that in the inclosure. All of the wheat in the general field, except that in a low and therefore

more fertile portion, remained erect throughout the season. The wheat in the inclosure was appreciably taller than was that under the glass or in the open field. Samples from the glass inclosure, from under the cover, and from the open were taken for analysis June 19. In taking a sample for analysis it was necessary to select some culms that were not entirely erect, for nearly all of them had partially or completely fallen. The results of the analyses of the oat and wheat culms are given in table XVII.

TABLE XVII

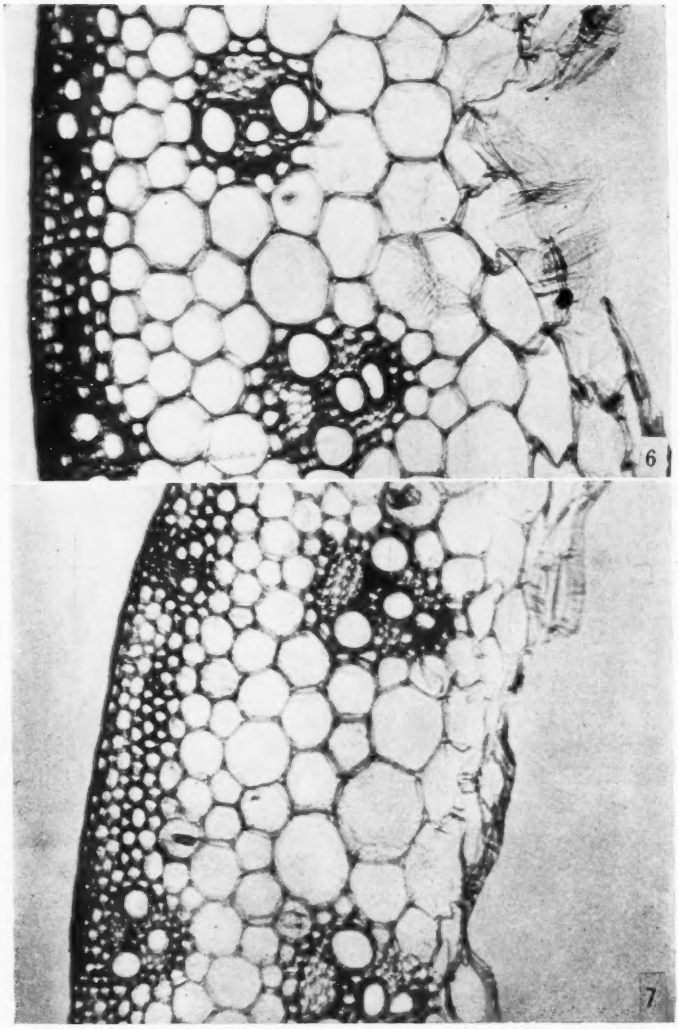
EFFECT OF TEMPERATURE ON PERCENTAGE COMPOSITION OF OAT AND WHEAT CULMS, 1924

Material	Oats			Wheat		
		Protected by glass			Protected by glass	
	Open	Cover	Inclosure	Open	Cover	Inclosure
Moisture	77.230	79.860	82.800	68.440	73.900	78.070
Dry matter	22.770	20.140	17.200	31.560	26.100	21.930
Nitrate nitrogen	0.002	0.002	0.002	0.001	0.001	0.002
Total nitrogen	0.103	0.114	0.097	0.051	0.065	0.059
Free reducing sugars	1.830	0.769	0.610	1.554	1.234	1.016
Disaccharides	0.378	0.450	0.319	8.959	5.735	2.062
Polysaccharides	4.283	3.398	3.035	6.194	4.943	4.849
Cellulose	3.412	3.386	3.202	4.292	4.301	4.239
Lignin	9.565	8.498	7.115	8.308	8.827	7.801
Total carbohydrates	19.468	16.501	14.281	29.307	25.040	19.967

From table XVII it may be seen that the plants grown in the inclosure contained a higher content of moisture, a lower content of dry matter, and a lower content of all the carbohydrate compounds. The greater contrast in the composition of wheat than in oats is probably due to the fact that the former was covered at a much earlier stage of development than was the latter.

ANATOMICAL

Cross-sections made on the stems show that the different environmental conditions affect materially the development of the various kinds of tissue contained in the culms. The effect on the structure of wheat culms of moderate differences in fertility, such as may be found in any field between hilltop and valley, is very



FIGS. 6, 7.—Oat culms: fig. 6, unshaded; fig. 7, shaded

marked. The thickness of the culm wall grown on high ground is usually about one-third greater, and the hypodermal zone is much more completely lignified, than in culms grown on more fertile low ground.

The effect of shade on the development of oat culms is shown in figs. 6 and 7. In the unshaded oats the width of the zone of parenchyma tissue is greater than in the shaded. Although the width of the hypodermal zones is about equal in the two, the quantity of lignified tissue is greater in the unshaded. In the shaded wheat culms the zones of parenchyma and hypodermal tissues are both somewhat greater than in the unshaded stems. More lignified tissue also is found in the unshaded culm. These differences are illustrated in figs. 8 and 9.

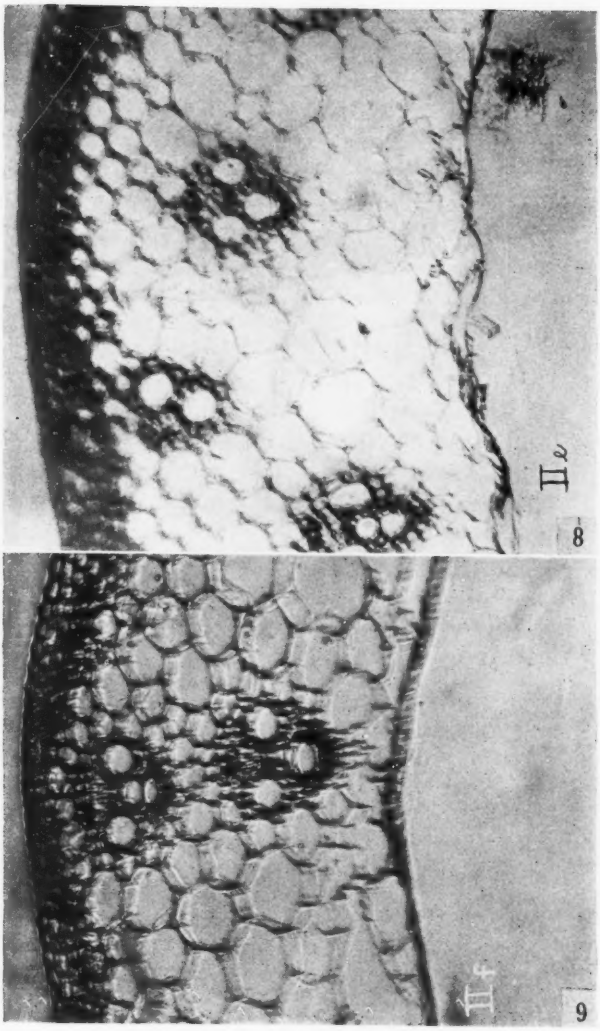
Rate of seeding also affects the structure of the culm. Stems taken from oat seedings made at the rates of 4 and 14 pecks per acre are shown in figs. 10 and 11 respectively. Although the total thickness of walls in the two seedings was practically equal, the width of the hypodermal zone is much wider in the thin seeding.

The difference in thickness of culm wall of wheat sown at the rates of 3 and 10 pecks per acre is shown in figs. 12 and 13 respectively. The total width of the culm wall was greater in the thin than in the thick seeding. Although the zone of hypodermal tissue was somewhat narrower, the cells were more completely lignified and compact.

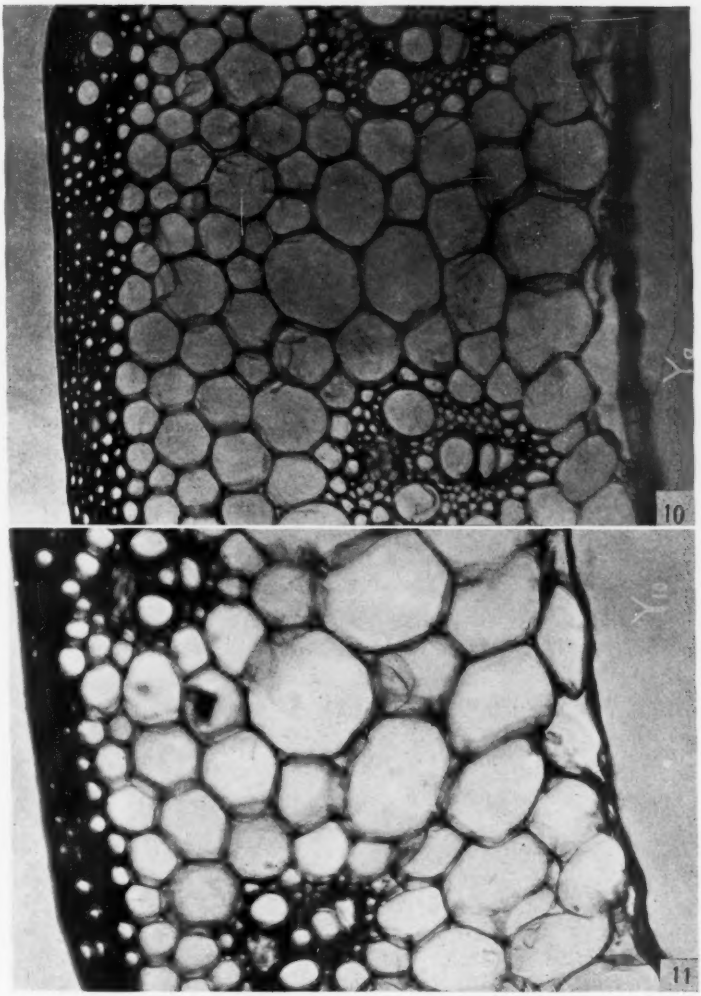
The effect of the use of large and small seed on the structure of oat culms was observed, and it was found that the total width of the culm was greater from the use of the large seed. The width of the hypodermal zone and the amount of lignification were also greater in the culm grown from the large seed.

Discussion

In field practice lodging is much more prevalent on rich than on poor land. Analyses of samples taken in 1924 from rich low land and from poor high land showed that the culms of oats and wheat taken from the former contained a higher moisture and a correspondingly lower dry matter content than did those grown on the latter. The plants with the lower dry matter content lodged.

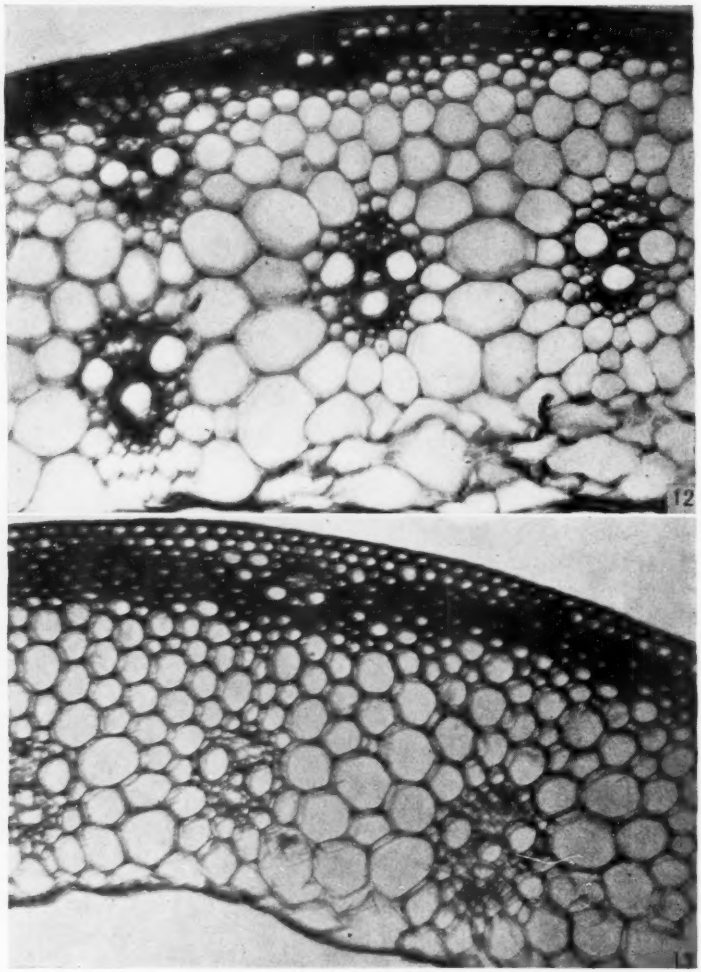


FIGS. 8, 9.—Wheat culms: fig. 8, unshaded; fig. 9, shaded



FIGS. 10, 11.—Oats: fig. 10, four pecks per acre; fig. 11, fourteen pecks per acre

In each of the three sand-soil-manure tests, analyses of the culms of oats and wheat showed that those grown in the manure contained a higher moisture and a correspondingly lower dry matter content



FIGS. 12, 13.—Wheat: fig. 12, three pecks per acre; fig. 13, ten pecks per acre

than did those grown in either the soil or the sand. The plants with the lower dry matter content lodged.

In the shading tests analyses of the culms of oats and wheat showed that those grown under the cheesecloth contained a higher moisture and a correspondingly lower dry matter content than did those grown in the open. The plants with the lower dry matter content lodged.

In the temperature tests analyses of the culms of oats and wheat showed that those grown in the inclosure contained a higher moisture and a correspondingly lower dry matter content than did those grown either under the glass cover or in the open. The plants with the lower dry matter content lodged.

From these experiments, therefore, the conclusion would seem to be warranted that lodging in oats and wheat results from high moisture and correspondingly low dry matter content of the culms. This is in accord with the findings of RIVERA (**29**), who, working with wheat, concluded that lodging was due to high water content of the tissue. The factors of which this situation is the index are, he says, the "true cause" of lodging.

The relatively low dry matter content of all the lodged culms was due to a relatively low carbohydrate content, particularly lignin, disaccharides, polysaccharides, and other reserve materials.

Of the various carbohydrates, the disaccharides and polysaccharides would probably be most likely to enter into the cell wall structures. In this connection it is of interest to note that in the low carbohydrate culms the zone of hypodermal or supporting tissue is in all cases relatively narrow. Furthermore, the cell walls are relatively thin and the amount of lignified material is reduced.

The low carbohydrate content of the lodged culms was due to various causes. In the shading experiments it was due apparently to a reduction in photosynthesis. The decrease in intensity of light from the use of the cheesecloth seemingly interfered with the photosynthetic processes, with the result that the total quantity of photosynthate manufactured was reduced.

In the temperature experiments, the low carbohydrate content of the culms grown in the inclosure was due undoubtedly to an acceleration of respiration. As is well known, the quantity of carbo-

hydrates present at any one time in plants represents the difference between two antagonistic processes, assimilation and dissimilation. Under the influence of relatively high temperature, therefore, when the processes of dissimilation would be accelerated, the residue of reserve material available for cell wall and other structures would be reduced. WILEY (40), WALSTER (38), and TOTTINGHAM (34, 35) found that low temperature favored an accumulation of carbohydrates.

In the sand-soil-manure test, the deposition of carbohydrate compounds in the plants grown in the manure was reduced by reason of the fact that much of the material was undoubtedly diverted by the excess nitrogen to form amino acids, nucleoproteins, and other nitrogenous compounds. On the other hand, in the plants grown in the sand there was a dearth of nitrogen with which to combine, and hence the carbohydrates were laid down as lignin, cell wall thickenings, and various reserve materials. These results are in accord with the findings of KRAUS and KRAYBILL (17), who, working with the tomato, found that plants grown in highly nitrogenous soil were characterized by a low content of dry matter, vigorous vegetative growth, and little reproduction; while those grown in poor soil or sand were characterized by a high content of dry matter, little vegetative growth, and poor reproduction. In this connection it may be said that in the sand-soil-manure tests there was always a preponderance of vegetative growth in the oats and wheat grown in the manure. Moreover, shading and high temperature also induced a preponderance of vegetative growth. In lodged plants there is usually a preponderance of vegetative growth, as evidenced by a relatively high percentage of straw, reference to which was made in tables I and II.

Since the strength of oat and wheat straw was found to vary with the carbohydrate content of the culms, it may be concluded that carbohydrates are an important integral factor governing the trend of development of oat and wheat plants with respect to straw strength or straw weakness. Also, important environmental factors which have a bearing on the development of carbohydrates in these plants are soil nutrients, light, and temperature. That the character

of growth made by many kinds of plants can be controlled, within limits, by the environmental conditions, was long ago pointed out by KLEBS (11, 12).

Summary

1. Lodging results from low dry matter per unit length of culm.

2. Low dry matter per unit length of culm may result from a relatively low composition of lignin and various reserve materials, such as disaccharides and polysaccharides, or from the development of relatively small slender culms.

3. Culms, small in diameter but medium or high in composition of carbohydrates, may result from direct causes such as thick seeding or the planting of small seeds. Such culms, however, exhibit a low dry matter content per unit length of culm.

4. Culms low in composition of carbohydrates are due to the development within the plant of a low carbohydrate nitrogen relation.

5. A low carbohydrate nitrogen relation may result from hypernutrition, shading, or relatively high temperature.

6. Under field conditions lodging results from the interaction of all these environmental factors. Hypernutrition not only tends to induce a greater elongation of the culms, but through a stimulation of the tillering processes, it increases the thickness of stand and hence results in a greater degree of shading around the base of the plants. In warm rainy cloudy seasons, therefore, the conditions are most favorable for the development of lodging on rich soils.

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OHIO AGRICULTURAL EXPERIMENT STATION
WOOSTER, OHIO

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VASCULAR ANATOMY OF RANALIAN FLOWERS

II. RANUNCULACEAE (CONTINUED), MENISPERMACEAE, CALYCANTHACEAE, ANNONACEAE

GEO. HUME SMITH

(WITH SIXTEEN FIGURES)

The vascular anatomy of the typical flowers of several genera of the Ranunculaceae has been studied by the writer, and the conclusion reached that the family is primitive (17). This inquiry into the fundamental nature of the Ranalian flower has been extended, in the present paper, to include certain unusual flowers of the genera *Ranunculus*, *Caltha*, *Delphinium*, and to include the families Menispermaceae, Calycanthaceae, and Annonaceae. Each of these groups shows additional evidence as to the nature of the Ranalian flowers.

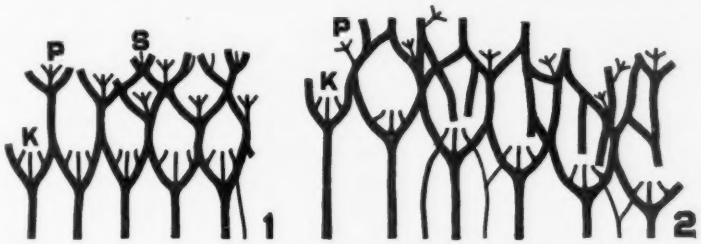
Ranunculaceae

In continuing the study of the Ranunculaceae, four variations from the usual floral organization will be considered. The first group includes those unusual flowers of *Ranunculus* in which the number of petals is more than five. For instance, during the past few seasons the writer has collected flowers of *R. abortivus* L., *R. acris* L., *R. recurvatus* Poir., and *R. septentrionalis* Poir. having more than five petals. Of these four species *R. septentrionalis* is especially prolific in the production of flowers with six, seven, or even eight petals, and in *R. acris* the variations are frequent. The second group includes the horticultural forms of *Ranunculus*. In these forms the flowers are completely "double"; that is, "the stamens and the carpels are completely replaced by petals." The third group considers a horticultural form of *Caltha palustris* in which "the stamens and the carpels are completely replaced by petals"; that is, the flower is completely double. The fourth group discusses horticultural forms of *Delphinium* in which the lowest stamens are replaced by petals.

1. FLOWERS OF RANUNCULUS WITH MORE THAN FIVE PETALS.—In the genus *Ranunculus* the flowers usually have five petals, but

individual flowers having six, seven, eight, or nine petals are not infrequent.

The vascular skeleton of a flower with more than five petals is represented by fig. 1. This figure is an actual tracing of the course of the vascular strands of a flower of *R. septentrionalis* with six petals, and can be taken as typical of any flower of *Ranunculus* having more than five petals. The axis is represented as split open and flattened out in one plane. The broader lines represent axial strands; the narrower lines represent the departing traces. The figure maps completely all strands of the sepal and petal region, and in addition



FIGS. 1, 2.—Fig. 1, *Ranunculus septentrionalis:* vascular system of axis of sepal (*K*), petal (*P*), and lower stamen (*S*) region split open and spread out in one plane; note presence of six distinct petals (*P*). Fig. 2, *Caltha palustris:* vascular system of completely double flower as if split open and spread out in one plane, showing sepal (*K*) and lower petal (*P*) region.

shows the first four stamens. If the higher levels were shown they would be the same as those pictured for the flower with the usual number of petals. The vascular skeleton of flowers having seven, eight, or nine petals differs from the flower portrayed in fig. 1 only in that a spiral consisting of a correspondingly greater number of petal traces exists.

The anatomical condition just described for these unusual flowers of *Ranunculus* suggests additional arguments to substantiate the contention that the petals and stamens of *Ranunculus* are closely related structures. For example, in the five-petal flowers of *Ranunculus* both petal and stamen are fundamentally single-trace appendages. In the flower with more than five petals, the petals and stamens are not only single-trace appendages, but also are arranged

in one continuous unbroken spiral series. They show, therefore, a striking unity of form and arrangement. Again, any suggestion that the additional petals are merely lobes of a fundamental five-petal condition, due to a complete splitting of one or more of the five petals, is not possible for several reasons. For instance, the trace to each petal departs from a different level and from a different point on the axial cylinder, and not in a progressive spiral succession and equally distant from each other. Another proof that each additional petal is a unit is deduced from the fact that each petal has a nectariferous scale at its base, regardless of whether the flower has five petals or more. Also it is to be noted that, whether the number of petals is five or more, each petal is supplied in identically the same fashion as every other petal; that is, each petal receives a single trace which arises directly from the axial vascular cylinder itself and which branches into three veins just as it enters the petal.

In conclusion, therefore, flowers of *Ranunculus* having more than five petals differ from the more usual five-petal flowers only in that the number of petals is variable (indefinite) and the insertion of the petals is spiral. As these two features, variability in the number of appendages and spiral arrangement of appendages, are indicative of a primitive floral organization, these unusual flowers represent a more primitive, a more *Caltha*-like organization than the usual five-petal flowers. Furthermore, a very close morphological relationship between petal and stamen is revealed by a study of the unusual flowers of *Ranunculus*, due to the fact that they are not only both single-trace appendages, but also are inserted in a continuous uninterrupted spiral sequence.

2. DOUBLE FLOWERS OF RANUNCULUS.—Horticulturists have succeeded in obtaining strains of *Ranunculus* in which the "stamens and the carpels are completely replaced by additional petals." Of these horticultural varieties, flowers of *R. septentrionalis* and *R. acris* have been studied. A reconstruction of the vascular skeleton of this type of flower displays one very important difference from a flower having several petals; namely, every trace above the sepal level, a total of sixty or more, instead of the first six to nine traces, partakes of the characteristics of petal traces; that is, each trace divides into three main veins as it passes through the zone transi-

tional between axis and appendage. This "double flower," therefore, either represents one in which both "stamens and carpels have been changed into petals," or one in which the stamens have been transformed into petals and the terminal carpellary region has been suppressed.

Four facts point to the conclusion that the carpels have been completely suppressed and all of the petals of these double flowers of *Ranunculus* are the homologues of stamens. The first is that each of the sixty or more appendages above the sepal level has a nectariferous scale at its base, just as each of the five petals of the ordinary flower has a nectariferous scale at the base. The second is that all appendages above the sepal level are supplied with the petal type of trace, namely, a single trace which branches into three veins. The third is that the total number of the petals of a double flower is approximately equal to the total number of petals and stamens of the usual flower of *Ranunculus*, rather than equal to the total number of petals, stamens, and carpels of the usual flower. The fourth is that, in the double flower, the series of departing traces is unbroken from the level at which the first petal trace arises until the axial vascular strands fade away as the last petal trace departs; whereas in the flower with five petals, and also in the flower with six to nine petals, a distinct interval exists between the point of departure of the last stamen trace and the first carpellary trace. Whether the axial interval between stamen and carpel in these flowers represents a single elongated internode or a region from which appendages have been suppressed does not enter into this question. The mere fact that the interval is not present in the double flower suggests that the petals of these flowers form one continuous unit, and the carpellary region has been suppressed.

In conclusion, therefore, the double flower of *Ranunculus* is one in which two modifications have taken place: (1) all of the stamens, not the lowest nine or less, have become transformed into petals; (2) the entire carpellary region has been suppressed.

3. DOUBLE FLOWERS OF CALTHA PALUSTRIS.—*Caltha palustris* normally has no petals, but a horticultural form exists in which the "stamens and the carpels are replaced by expanded petal-like appendages." In color and shape these appendages are similar to se-

pals, but they are decidedly smaller. The outermost are about one-fourth the size of the sepals, and they gradually diminish in size until the innermost are very small. Even the innermost are not tipped with pollen sacs, and therefore no transition from petal to stamen can be traced, as is possible, for example, in *Castalia*.

The basal portion of the vascular skeleton of a "double flower" is portrayed in fig. 2. As in the normal flower of *Caltha*, traces depart from the axial vascular cylinder, so that each sepal is supplied with three traces. Then, continuing the spiral, traces depart to the petals, each of which receives one trace. Just as this trace passes out of the receptacle into the petal it branches into three prongs, forming the three principal veins of the petal. As the apex of the receptacle is approached, the axial strands rapidly diminish in size. For instance, a cross-section of the axis at 1000 μ from the apex reveals that each axial strand contains eight or more lignified elements. At 600 μ the strands have decreased to about a third of their former dimensions, and the lignified elements have decreased to two or three in each strand. At 400 μ the strands are entirely devoid of lignified elements, and are scarcely distinguishable from the surrounding parenchyma. A section at 200 μ from the apex discloses no circle of bundles. The tissue is homogeneous parenchyma. Twenty or more petals arise above this point and hence are without a vascular supply. This condition is directly the reverse of the wild form, in which the remains of a vascular supply to completely suppressed terminal carpels exist; whereas in the double flower the axial vascular supply gives out before the uppermost appendages (homologues of stamens) are supplied, and all evidence of a carpellary region has vanished.

In comparing the vascular skeleton of the double flower of *Caltha* with the uncultivated apetalous form, three striking similarities between the two types appear. The number of sepals and stamens of the one approximately equals the number of sepals and petals of the other. This similarity and the two following, therefore, both argue for the homology of the petals of the double flower of *Caltha* and the stamens of the apetalous uncultivated flower. The second similarity between the two flowers is that the appendages in both cases are inserted in a spiral. The third similarity between the two

flower types, and incidentally a third argument for the similarity of petals and stamens, is that each sepal in both types of flower receives three traces, and each petal of the one flower and each stamen of the other flower receive a single trace.

On the other hand, the vascular skeleton of the double flower of *Caltha* differs from that of the common apetalous flower in two respects. In the first place, the double flower has no equivalent of the carpellate region of the normal apetalous flower. Three reasons already have been stated to show that the petals of the double flower are the equivalent of the stamens of the apetalous flower, and certain additional facts can be presented to substantiate this assertion. It was obvious in the study of the normal apetalous flower (17) that the more terminal carpels had disappeared, and it is obvious in the double flower that the axial bundles rapidly diminish in size as the apex of the receptacle is approached, and thus indicate that a considerable portion of the receptacle is suppressed. In the second place, the double flower of *Caltha* differs from the apetalous flower in that each trace passing to an appendage above the sepal level branches into three main veins, just as it passes through the parenchyma transitional at the point of union of the petal and receptacle, whereas in the wild apetalous form the conditions are different. The trace to the stamen passes as a single unbranched unit the entire length of the appendage, and at the higher levels the traces depart in groups of three as dorsal and ventral traces to the individual carpel.

In conclusion, therefore, the vascular skeleton of the horticultural form of *Caltha palustris* is fundamentally that of the wild apetalous form, from which it differs in but two respects: (1) the stamens have become transformed into expanded petal-like structures similar to the petals of *Ranunculus;* (2) not only has vascular supply to terminal suppressed carpels disappeared, but even the entire carpellate region and also the vascular supply to the uppermost petals (homologues of stamens) has disappeared.

4. INCOMPLETELY DOUBLE FLOWERS OF DELPHINIUM.—The vascular structure of the incompletely double flower of *Delphinium* cannot be understood until the structure of the normal flower has been described. In this flower (5 sepals, 4 petals) the three traces to each

sepal arise from separate and distinct points on the axial vascular cylinder, and three separate and distinct gaps in the axial vascular cylinder are formed. Above this level the axial strands subdivide and recombine, so that the cylinder becomes the usual network studded with departing traces. The traces of the petal-stamen region emerge from this network in a suggestively spiral fashion. Each trace that departs from the axial cylinder within this region becomes the entire supply to an appendage. The first four appendages above the sepal on the adaxial side are the petals, whereas the appendages of the same turn of the spiral on the abaxial side are stamens; that is, petal and stamen form one continuous series. As in *Ranunculus*, *Aquilegia*, and *Caltha* (double flower), the trace to a petal branches into three parts as it passes through the parenchyma transitional between petal and receptacle, whereas the stamen trace is entirely unbranched (fig. 1). After the stamens are supplied the axial strands become three; each of these strands becomes the supply to a carpel. In the basal part of each carpel the strand branches into its component three traces, one dorsal and two ventral.

The vascular structure of the double flower differs from the usual flower in only one respect. Each of the twelve to fifteen appendages above the sepal level has not only the external appearance of petal, but also receives the typical petal type of vascular supply, namely, a single trace which branches into three veins as it enters the base of the petal; whereas in the usual flower only the first four appendages on the adaxial side have the appearance and vascular supply of petals. In such other important respects as the combined total of petals and stamens the two flowers are the same; consequently, following the type of argument used in the discussion of the double flowers of *Ranunculus*, it can be proved that the petals and stamens of *Delphinium* form one continuous unit and are fundamentally similar.

In conclusion, therefore, it can be said that the vascular anatomy of the double flower of *Delphinium* illustrates and substantiates all of the conclusions drawn from the vascular structure of the preceding three groups.

Discussion

In reviewing the data presented by the unusual forms of the Ranunculaceae, additional evidence is brought forth as to the nature

of the petal. For instance, the vascular anatomy of these flowers shows that every petal is a single unit. The details of this argument were presented in the discussion of the flowers of *Ranunculus* having six or more petals.

The facts also demonstrate that the vascular supply to the petal displays an individuality of its own. In the ordinary flower of *Ranunculus*, *Aquilegia*, and *Delphinium* it was noted that every petal trace branches into three veins as it enters the base of the petal. In this study it is shown, for instance, that every flower of *Ranunculus septentrionalis*, whether the petals be five, several, or many, has this type of supply to each of its petals. The same can be said of *Delphinium;* and each of the sixty or more "petals" of *Caltha* are so supplied. The conclusion, therefore, is that this is a distinctive anatomical characteristic of petals whenever they occur in such members of the Ranunculaceae as *Ranunculus*, *Aquilegia*, *Delphinium*, and *Caltha*.

The question now arises as to whether the petals of these genera are distinct morphological units, morphological units closely related to the sepals, or morphological units of staminal affinity. The petals cannot be sepals, because of the difference in method of vascular supply. This difference is most clearly illustrated in the double flower of *Caltha*, *Ranunculus*, and *Delphinium*. Each sepal is supplied with three distinct traces, each petal with a single strand. If the single strand represented three laterally fused traces, some transitional stages certainly would have been observed in some of the flowers examined, but such have not been found. Thus no similarity to sepals exists. On the other hand, each of the forms studied gives evidence of the staminal affinity of the petals. The first is that the vascular supply to a petal, as that of a stamen, arises as a single trace. Again the axial vascular skeleton of the petal region of the double flower of *Caltha* has the exact ground plan of the stamen region of the single apetalous wild flower. Similarly, in *Delphinium* the stamen-petal region is a single unit. Also in *Ranunculus* the vascular skeleton of a flower having many petals presents the same plan as the petal-stamen region of the flower having six to nine petals, and this in turn is very similar to the flower having a whorl of five petals and an indefinite number of spirally arranged stamens. It seems obvious, therefore, that stamens and petals are closely related organs.

The evidence just presented to show the staminal affinities of petals also more closely links *Ranunculus* to *Caltha* than before. In the six-nine petal flower it is to be recalled that the insertion of the petals is spiral, not cyclic. It is also established that these petals are stamens; therefore the *Ranunculus* flower of several petals is in reality a flower composed of five whorled sepals, several spirally inserted modified stamens (petals), and an indefinite number of true stamens and carpels. It is also known that, in general, the indefinite number of parts has given rise to the definite number, and the spiral to the cyclic. If one pictures such changes as these in the organization of the calyx and the corolla of *Ranunculus*, and considers all stamens as unmodified, then *Ranunculus* is transformed into the more primitive *Caltha* type.

These forms also present evidence to show that the vascular tissue may disappear before the organ to be supplied. It will be recalled that in the double flower of *Caltha*, the vascular axial cylinder fades away before the uppermost and smallest petals (homologues of stamens) are supplied, whereas in the more typical apetalous form not only are all of the appendages supplied, but remnants of vascular supplies to apical and suppressed carpels exist.

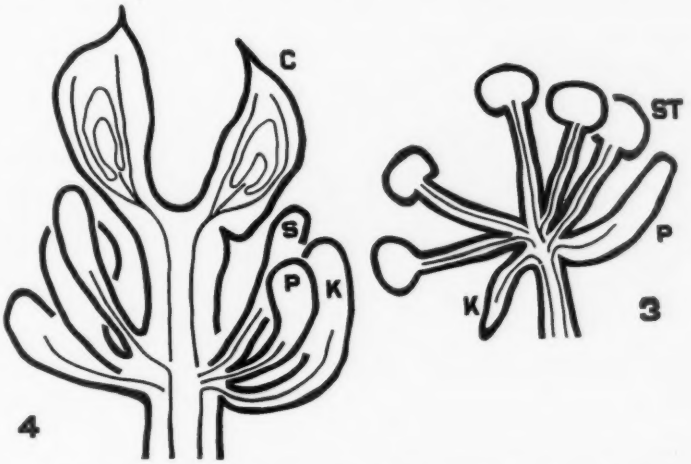
The unusual flower of the Ranunculaceae, therefore, whether partially or completely double, is built upon the same general plan as the normal flower; and when completely double, as in *Ranunculus* and *Caltha*, the carpellate region is totally suppressed. Furthermore, these unusual flowers show that all petals are modified stamens because of their single trace character, their formation of the continuous spiral with the stamens, their complete replacement of stamens of the double flowers in *Caltha* and *Ranunculus*, and the partial replacement in *Delphinium*.

Menispermaceae

If *Menispermum canadense* L. is taken as typical of the Menispermaceae, then the flower of this family shows certain distinct variations in vascular anatomy from the Ranunculaceae. Because of these variations the vascular anatomy of the flower is of interest in determining the nature of the Ranalian flower.

ANATOMY OF FLOWER.—In the staminate flower of *M. canadense* (fig. 3) the pedicel usually contains five separate and distinct strands.

At the sepal level these divide radially. Of the strands which result from this radial division some remain axial, and others supply the four to eight sepals with one trace each. Immediately thereafter these axial strands redivide and supply the petals in a like manner; then the remaining axial strands divide into a number of traces equal to the number of stamens. Occasionally, however, stamens which appear to be perfectly normal contain two distinct traces throughout their entire length. No special significance can be attached to this



FIGS. 3, 4.—*Menispermum canadense:* median longitudinal section of staminate flower (3), and of pistillate flower (4); *K*, sepal; *P*, petal; *St*, stamen; *S*, staminodium; *C*, pistil.

abnormality. The vascular framework of the staminate flower (fig. 3) thus displays a very marked resemblance to the staminate flower of *Thalictrum dioicum* of the Ranunculaceae.

In the fertile flower (fig. 4) the sepals, petals, and staminodia (nonfunctional stamens and usually six in number) are supplied in a manner similar to that for the corresponding appendages of the staminate flower. The axial cylinder then continues upward as a group of more or less distinct strands. This portion of the axis forms the stalk, upon the distal end of which are the two to four carpels. At this point the strands arrange themselves into as many groups

as carpels, and then each group enters a single carpel. Sometimes these groups appear to be composed of four distinct strands; at other times completely united into a single strand. Within the base of each carpel one strand proceeds to the stigma along the dorsal margin; two others toward the stigma along the ventral margin; and the fourth, which is located between the two ventral strands, directly to the ovule.

DISCUSSION

The carpels, as the anatomical evidence discloses, are of the typical three-trace type. They differ from the more primitive *Caltha* carpel, however, in that only one ovule is present; all vestiges of additional ovules have been lost. A second difference from the Ranunculaceae is that the strand to the ovule frequently becomes a separate entity before the carpel supply leaves the axial cylinder. This precocious splitting of the carpellary strand, however, is of no particular significance.

The stamens are normally single-trace appendages. Thus their origin still remains to be solved. The literature of floral vascular anatomy (1–20) consistently reports stamens to be single-trace appendages.

The staminodia of the fertile flower are probably the homologues of stamens for several reasons. The evidence is not direct, but several circumstantial facts point in this direction. The sepals and petals of the fertile flower correspond to similar appendages of the staminate flower; hence the staminodia appear to be an additional group of appendages. The stamen-like external appearance of these appendages is another possible indication of a stamen affinity. Likewise the single trace condition is a stamen characteristic, but cannot absolutely be diagnostic because, as is discussed in the following paragraph, petals and sepals are likewise single-trace appendages, but probably of a different origin. Finally, the appreciable axial interval between the point of attachment of the staminodia to the receptacle and that of the carpels suggests that intervening appendages (additional stamens) have completely disappeared. The origin and development of such a condition are easy to picture, when one recalls the conditions of *Aquilegia canadensis* (17). In this flower the upper-

most whorls of stamens, although supplied with vigorous traces, are small, chaffy, and have undeveloped anthers. They are evidently on the way to complete atrophy, and the fertile flower of *M. canadense* can be taken as a very advanced stage of this tendency. A somewhat intermediate step might be cited in *Ulmus*, in which BECHTEL (4) found that some of the stamens had disappeared but the traces to these stamens still existed.

The petals and sepals are also single-trace appendages, consequently two views as to the nature of the petal are possible: petals may be considered as non-functional stamens or they may be considered as sepals. Similarly two views are possible as to the nature of the sepals: they may represent a group of modified petals and thus represent a secondary modification of stamens, or they may be considered as modified foliage leaves. In the latter case they have been derived from a more primitive multitrace type of foliage leaf, either by a loss of the lateral traces or a fusion of the lateral traces to the median to form a single strand. Thus the fact that sepals, petals, and stamens are all single trace appendages, and are in one continuous series, obscures the affinity or lack of affinity of these appendages for each other.

It is also to be pointed out that *Menispermum canadense* presents evidence to show that imperfect flowers have been derived from perfect flowers. In the derivation of the flower having only stamens the chain of evidence is very clear. For example, in an earlier portion of this paper it was noted that in the wild form of *Caltha palustris*, vascular tissue passes toward apical and totally suppressed terminal carpels; and that in the horticultural form in which petals have replaced the stamens, not only have the carpels disappeared, but also the vascular conditions indicate that the stamen-bearing portion is fading away. Thus, similarly, it is possible to consider that the staminate flower of *Menispermum* is the result of the complete disappearance of the apical carpellary, and even possibly part of the staminal region. As regards the fertile flower, the presence of at least six stamen-like filaments, an appreciable interval, and then the carpels suggests that intervening stamens have disappeared. This can be asserted in spite of the fact that no vascular tissue passes

outwardly within this interval toward suppressed stamens, because it is entirely possible for both an organ and its vascular supplies to disappear simultaneously.

If *Menispermum canadense* is typical of the Menispermaceae, therefore, the vascular anatomy of the flower of this family is of the same fundamental design as that of the Ranunculaceae, and is a specialized offshoot from the more primitive generalized *Caltha* type. In this specialization the anatomical facts point toward two modifications: (1) the flowers have become differentiated into purely staminate flowers and into perfect flowers with the stamens nonfunctional, through the disappearance of both appendages and traces; (2) the sepals, petals, and stamens are similar, in that all are single-trace appendages. It has been impossible to determine whether this similarity does or does not bespeak a morphological affinity.

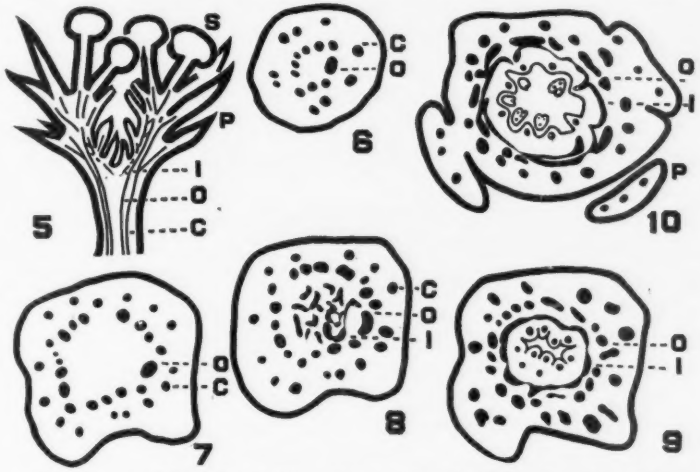
Calycanthaceae

In the Calycanthaceae the flower differs in form from the other families of the Ranales. Instead of the usual convex shape, the receptacle is deeply concave (fig. 5); in other words, the receptacle is inversely conical or cup-shaped. The carpels are placed upon the bottom and inner surface of the cup, the stamens upon the rim, and the perianth parts upon the outside. Because the shape of the flower is unusual, the distinctive features of the vascular anatomy are of particular interest in determining the relationship of this family to the other Ranalian groups.

ANATOMY OF FLOWER.—The vascular framework of the flower of *Calycanthus floridus* L. is sufficiently typical to represent the entire family. The peduncle contains two concentric cylinders of vascular strands (figs. 5, 6). The cortical cylinder (*C*) is composed of about ten distinct strands, and the axial or outer cylinder (*O*) of about ten or fifteen strands. Upon reaching the region at which the first few perianth parts arise, the strands of the cortical system are increased slightly in number by radial divisions (fig. 7). These strands then pass upward and outward and enter the first few scattered perianth parts. Three or four adjacent strands enter a single perianth part. Thus the cortical cylinder of vascular tissue vanishes.

Next the course and the changing characteristics of the outer

cylinder of vascular strands will be followed from the peduncle upward to the rim. Slightly below the level at which the first group of strands from the cortical cylinder disappear into perianth parts, strands of the outer cylinder divide radially (fig. 7). Some of the resulting strands move outward and upward, and in turn enter higher perianth parts. The majority of the strands, however, remain in



FIGS. 5–10.—*Calycanthus floridus:* fig. 5, median longitudinal section of flower; figs. 6–10, cross-sections of flowers at successively higher levels; *C*, cortical cylinder of vascular tissue; *I*, inner cylinder of vascular tissue (inverted portion of outer cylinder); *O*, outer cylinder of vascular tissue (upright portion of axial cylinder); *P*, perianth part; *S*, stamen. Carpels seated on interior of cup.

alignment with the outer axial cylinder and continue to divide radially. At each of the divisions of an axial strand some of the segments become perianth traces, others remain as axial strands. Before traces have departed to many perianth parts the individuality of the strands of the outer cylinder gradually becomes obscured. This is due to the oblique passage of numerous elongated parenchymatous cells and spiral xylem elements from one upright axial strand to the next (fig. 10). As the rim of the receptacle is approached the outer cylinder again breaks up into distinctly individual strands. Some of these pass outward in groups of three or four to the uppermost perianth parts; others pass inward to the stamens which are

seated upon the inner rim of the receptacle. One trace enters each stamen. Thus a cross-section of a flower at the rim of the cup reveals groups of three or four vascular strands in various stages of outward upward passage to perianth parts, and also single strands passing inward, upward to stamens (fig. 5).

Such sections also reveal the interesting fact that a third zone of vascular tissue, inner cylinder (*I*), exists (fig. 10). The significance of this zone can be understood best by tracing its passage downward and inward from the rim of the cup toward the central basal portion of the receptacle. The strands of this cylinder can be distinguished from the strands of the outer cylinder because the xylem elements of the strands of the outer zone are in the normal centripetal direction, whereas the xylem elements of the strands of the inner zone are centrifugal. Furthermore, these bundles are very closely appressed to the vascular tissue of the outer zone for some distance downward. Frequently actual unions between the two cylinders occur (fig. 10). Only gradually do the strands of the inner zone converge toward the central basal portion of the receptacle (fig. 5). In the downward course of the strands of this cylinder, traces depart inwardly to the carpels. First the dorsal and then the two ventral traces depart to the carpel. The departure of the pairs of ventral traces accounts for the scalloped appearance of the inner cylinder of vascular tissue in fig. 10. The farther downward one follows the inner cylinder the less distinctly individual become the upright strands. This is due to the spreading out laterally of the spiral elements of one vertical strand toward the next. The cylinder thus becomes outlined as an interlacing mass of xylem elements. After the traces to the last carpels have departed and the receptacle has become a solid structure, the vascular elements of the inner cylinder become fewer in number, less lignified, and converge into a small group of elements in the basal central portion of the receptacle (fig. 8), and then finally disappear (fig. 7).

The course of the three traces which enter a carpel is very definite. One passes up the dorsal margin of the carpel, and the other two pass up the ventral margin as two distinct traces. If two ovules are present, each ventral trace gives rise to an ovular strand; if only one ovule appears, but one ventral trace puts forth an ovular strand.

Discussion

Carpels.—The study of the anatomy of the carpels of *Calycanthus* reveals two interesting facts. The vascular structure is such that these carpels represent a condition intermediate between the three trace multiovulate carpel of *Caltha* or *Trollius* and the single strand (three traces united) uniovulate carpel of *Ranunculus* and *Hepatica*. It will be recalled that in the study of the Ranunculaceae (**17**) the multiovulate carpels of the *Caltha-Trollius* group displayed vascular conditions suggesting the gradual elimination of the apical ovules. *Calycanthus* would represent this tendency carried to the extent that all of the ovules but one or two have disappeared. The second interesting fact is that the ventral suture of a carpel is plainly visible, even after the ovules are well formed. In early stages of a carpel's development the two ventral margins can be found to be in close contact but not actually fused, except the upper third. The union of the ventral margins occurs rather late in the ontogeny of a carpel in this form.

Stamens.—Each stamen receives a single trace. The literature of floral vascular anatomy (**1-20**) likewise records stamens as uniquely single-trace appendages.

Perianth.—The vascular structure of the perianth differs from that of the majority of the Ranunculaceae in two respects. The first difference is that the number of traces to a single appendage is either three or four, whereas among the Ranunculaceae the number is more typically three. However, *Clematis* frequently has four traces to a sepal. The second difference is that the perianth parts of *Calycanthus* range from the lower scattering bract-like structures through sepal-like structures to colored petal-like structures, all receiving the same type of vascular supply. Therefore the petals of the Calycanthaceae, if one calls the upper colored perianth parts petals, are distinctly sepaline in nature, whereas the petals of the Ranunculaceae are distinctly staminal in affinity.

Receptacle.—The description of the receptacle of *Calycanthus* has revealed four interesting differences from the condition found in the Ranunculaceae. The first is that the receptacle is cup-shaped. The second is that the perianth parts are inserted on the outside of the cup, the stamens on the rim, and the carpels upon the inside.

The third is that the dorsal trace to a carpel departs from the vascular cylinder at a higher level than the two ventral traces. The fourth is that the upper portion of the receptacle contains two concentric systems of vascular tissue. Of these two systems the outer has the xylem tissue oriented toward the center, and the inner has the xylem tissue toward the outside. It is also to be noted that the two systems are not directly connected to form a continuous unit.

How could a floral primordium develop to fulfill these four conditions? If it is supposed that the rate of growth of the cells forming the apex of a normally convex floral primordium be checked (all portions of a meristematic region do not grow at the same rate), and the ring of meristematic tissue surrounding the very apex should continue to bulge up and grow, then the apex of the floral axis would become overshadowed by the bulging ring of tissue. As a result the floral appendages would spring from the outer and the inner surfaces of this overarching portion of the axis. The perianth parts would spring from the outer surface, the stamens from the rim, and the carpels would arise nearest the true apex, that is, from the interior of the cup. Consequently they would seem to be upside down. For this reason the dorsal trace does seem to arise at a higher level than the two ventral traces, that is, it arises nearer the rim, pseudo-apex of the cup. Again, because of the bulging of the meristem surrounding the apex, the axial vascular tissue would follow an inverted V-shaped course, and consequently the xylems of the upright and recurved portions would be facing each other. An actual union between the outer upright and inner recurved zones might be lacking and could be explained as follows. The close proximity of the two vascular zones at the point of sharp bending downward of the vascular tissue has resulted in frequent secondary unions, as mentioned in the description and noted in figs. 5 and 10. Such would furnish a ready and efficient channel of transportation between carpel and peduncle, so that the development or absence of the vertex of the inverted V would be of no physiological consequence, and its disappearance would not affect the plant's activities. Thus all of the unusual features of the vascular anatomy of a flower of *Calycanthus* can be explained upon the basis of a checked apical growth of the floral primordium.

PHYLOGENETIC POSITION.—In the description of the wild uncultivated form of *Caltha palustris* (17) it was shown that the frayed termini of the axial strands wander toward the apex of the conic receptacle; the floral appendages are indefinite in number and spirally inserted; the carpels are three-trace appendages; the stamens single-trace appendages; and the sepals three-trace appendages (but in the closely related *Clematis* either three or four traces). If one will picture the vascular skeleton of such a flower of the *Caltha* group of the Ranunculaceae with the apex of the receptacle indented, as one would push in the finger of a glove (due to the arrested growth of the apical primordium), one duplicates the vascular skeleton of the flower of the Calycanthaceae; therefore the Calycanthaceae are a specialized offshoot of the Ranunculaceae in which the growth of the apical primordium of the flower is delayed.

ORIGIN OF INFERIOR OVARY.—The study of *Calycanthus* arouses interest in a further consideration of the inferior ovary. Morphological and taxonomic evidence points to the inferior ovary as having arisen from several different centers of development. Has the inferior ovary always arisen after the same fashion? From the standpoint of vascular anatomy, such records as do exist (together with the author's own unpublished data upon the vascular anatomy of *Potentilla*, the cultivated roses, and *Sassafras*) indicate that the inferior ovary is due to a complete adnation and cohesion of the outer floral appendages to one another and to the ovary. *Calycanthus*, on the other hand, suggests the possibility that the inferior ovary could arise, due to a checking of the growth of the apical portion of the floral primordium, a bulging up of the surrounding meristematic tissue, and a subsequent fusion of this overhanging axial tissue with the carpel. Therefore the problem of the mode or modes of formation of the inferior ovary merit further and careful investigation from the standpoint of vascular anatomy.

CORTICAL VASCULAR SYSTEM.—The cortical vascular system of the flower differs from the cortical vascular system of the stem in several respects. For instance, the cortical bundles of the stem of *Calycanthus* (21) are inversely oriented, whereas those of the flower are not. Again the leaves and the leaf traces are arranged oppositely, whereas the floral appendages and floral traces are spirally

arranged. A third difference is that all of the traces to the lowermost perianth parts enter into the cortical system, but in the case of leaves the two lateral traces drop down through the cortex and the median leaf trace enters directly into the axial cylinder. The fourth difference is that all the traces from the higher perianth parts drop down through the cortical region slightly before passing into the vascular cylinder. Thus the traces from floral parts fail to form a continuous cortical system, as all of these traces ultimately pass into the axial cylinder; whereas in the vegetative part of the plant only the median leaf trace enters the axial cylinder, and the lateral leaf traces form a definite, continuous cortical system.

Whether the cortical vascular system of this floral axis represents a development independent of that of the vegetative axis (a less highly developed stage of such a development) or the remnants of such a condition cannot be determined; nevertheless a cortical vascular cylinder, in addition to the axial cylinder, denotes a derived condition. Therefore additional evidence is at hand to show that the flowers of the Calycanthaceae are a very specialized offshoot of a more primitive type, namely, the Ranunculaceae.

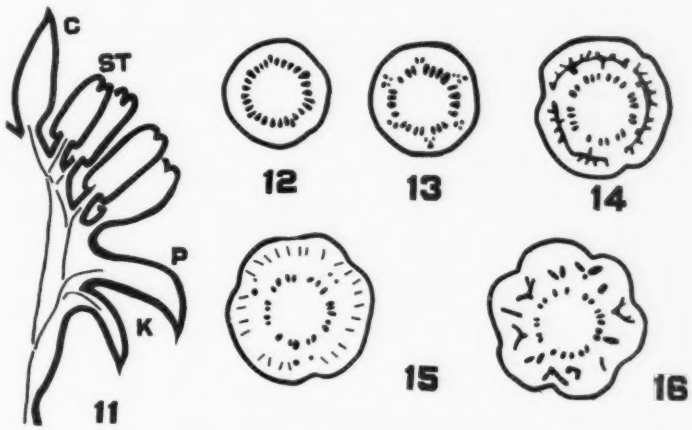
A summary of the preceding points is as follows. (1) *Calycanthus* has become specialized from forms having numerous perianth parts morphologically indistinguishable from one another, each receiving three or four traces, numerous single trace stamens, numerous three-trace carpels, and all appendages inserted in a strictly ascending spiral. (2) A suggestion of a cortical vascular system is present. (3) The hollow cuplike shape of the flower is due to a checking of the growth of the apical primordia, thereby giving the flower its inversely conic shape. (4) *Calycanthus* gives indication that the inferior ovary could be derived through a checking of the growth of the apical primordia of the receptacle, accompanied by a consequent bulging up of the surrounding meristematic tissue and a subsequent fusion of this overarching tissue with the ovary wall. On the other hand, the literature of floral vascular anatomy hints that the more common method is by adhesion and cohesion of floral appendages to one another.

Annonaceae

In the groups of the Ranales already investigated (Ranunculaceae, Menispermaceae, and Calycanthaceae) the vascular skeleton

is fundamentally of the same character. Does *Asimina triloba* Dunal. show the same characteristics of floral vascular anatomy as the three families just mentioned?

ANATOMY OF FLOWER.—In the pedicel the axial cylinder of vascular tissue consists of about thirty small wedge-shaped strands (fig. 12). In the lowest part of the receptacle some of the strands depart from six to eight points on this cylinder of small wedge-shaped strands toward the periphery. Usually six of these points are op-



FIGS. 11–16.—*Asimina triloba:* fig. 11, median longitudinal section of flower; figs. 12–16, cross-sections of flower at peduncle (12), sepal (13–15).

posite the median radii of the six petals. Either one small wedge-shaped strand departs from a point, or a group of two to four strands depart (fig. 13). No consistent plan is followed. As these strands pass upward and outward they sometimes gradually reverse their orientation. The change is most pronounced where groups of two to four strands leave the axial cylinder at one point. As these groups approach the sepal level they spread laterally in the cortical region of the receptacle, and fuse so that three arcs of tissue are formed (fig. 14). These arcs then break up into individual strands, all of which, except the end ones, pass outward as the traces to the sepals (fig. 15), thus forming the entire vascular supply to the first whorl of perianth parts. The strands forming the two extreme portions of

these arcs bend upward to form six to eight distinct cortical strands (fig. 15). Six of these are always on radii alternate with the petals, and at the petal level subdivide, so that the majority of the resulting strands pass outward and enter the six petals as the lateral traces to these appendages (fig. 16). The anatomical evidence indicates that all six petals are inserted at one level, rather than in two whorls of three each. The remaining cortical strands advance upward to higher levels; then some pass outward and enter the stamens, one to a stamen (fig. 11); others orient themselves so that they tie together the axial cylinder and the cortical vascular tissue (fig. 11). The petals, it has been stated, receive their lateral traces from the outer cortical cylinder, and they receive the median traces directly from the axial cylinder of small wedge-shaped strands. Thus the petals receive numerous traces; a portion of these are derived from the cortical cylinder and a portion from the axial cylinder. Some of the lower stamens receive their supply from the cortical cylinder, whereas others receive theirs from the axial cylinder of small wedge-shaped strands (fig. 11). A strand which passes outward from the cylinder toward a stamen may pass unbranched to a stamen, or may branch within the cortical region of the receptacle into from two to four strands and each strand pass to a stamen as its entire supply. Within the stamen a trace passes unbranched up the filament and connective. As it approaches the tip of the connective it flares into a fan-shaped series of strands and disappears. The orientation of some of the stamen traces which pass an appreciable distance upward through the receptacle becomes partially or entirely inverted. Above the stamen level the vascular cylinder of wedge-shaped strands breaks up into unit groups of three strands each; these units enter the individual carpel as one dorsal and two ventral traces. Within the carpel the dorsal trace sends forth a few lateral branches as it advances to the stigmatic region. Each ventral trace gives rise to an individual strand to each ovule of the row beneath it, and at the same time gives rise to a few dorsally directed branches.

Discussion

The vascular system of the floral axis of *Asimina triloba* shows certain features akin to the cortical vascular system of a vegetative

axis. Many of the stamen traces drop down through the cortex and join other stamen traces at lower levels, just as leaf traces may drop down through the cortex. It is this condition which leads to the misconception that "a trace which passes outward from the axial vascular cylinder may branch within the cortical region of the receptacle, into from two to four strands and each strand pass to a stamen as the entire supply to that stamen." The condition is rather the reverse; several separate traces are fused to form a single cord. An even more striking likeness to the cortical vascular system of a vegetative axis is found in the petal region of the flower. The median petal traces pass directly to the axial cylinder, whereas the lateral petal traces drop down through the cortex and join lateral sepal traces, just as lateral leaf traces may drop down and unite with other lateral leaf traces. Here the similarity ceases. All of the sepal traces, both median and lateral, drop down through the cortex, but ultimately enter the axial vascular cylinder, thereby terminating the cortical system. It is also interesting to note that some of the cortical strands reverse their orientation as they drop down through the cortex. This again is a feature of cortical vascular systems. Thus a tendency, although not uniformly expressed by all of the individual floral appendages, exists toward the formation of a cortical vascular cylinder of tissue. A study of other members of the family should add more interesting data.

Since a tendency toward the formation of a cortical vascular system also exists in the flower of *Calycanthus floridus*, comparisons with that flower are interesting. In *Asimina* all perianth traces ultimately enter the axial vascular system, whereas in *Calycanthus* the trace to all of the lower perianth segments enters the cortical system, and all of the traces to the upper perianth parts enter the axial cylinder. Thus, in addition to the fact that the shape of the flower differs, the two cortical vascular systems have no definite common characteristics. This would indicate that the two cortical vascular systems have developed independently, and it may also indicate that the perianths of *Asimina* and *Calycanthus* are not homologous structures. The study of other members of the family should throw further light upon this subject.

The carpels reveal no conditions not already discussed in the

Ranunculaceae. They are of the primitive three-trace multiovulate type.

The stamens, as in all of the other families studied, are distinctly single-trace appendages. One peculiarity exists, however, which makes it desirable that other members of the family be studied. As has been said, some of the stamen traces pass directly inward to the axial cylinder, and others drop downward and join other stamen or petal traces, thereby forming a cortical vascular system. It was first thought that a regular alternation of axial and cortical traces existed. If it had been found that the stamens were so grouped that a stamen having a trace passing directly to the axial cylinder was flanked on each side by a stamen with its trace passing to the cortical system, then the situation would have been interesting. In the first place, every group of three stamens could have been taken to be a single unit, analogous to many vegetative axes in which the lateral traces of a leaf are cortical and the median trace is axial. In the second place, it still remains to be proved that every stamen with its anther and filament represents all of a single sporophyll. In the third place, evidence is accumulating that in one and perhaps a second of the remaining families of the Ranales three-trace and therefore presumably primitive stamens do exist. Thus the mere fact that an irregularity in the behavior of stamen traces of *Asimina triloba* does exist suggests that a further investigation of the family is desirable.

The petals of *Asimina* differ from the petals of the Ranunculaceae in two respects. They receive numerous traces, and the lateral traces are derived from a cortical vascular system. The first difference, numerous traces, definitely removes any staminal affinity, whereas the strictly single-trace condition of the petals of the Ranunculaceae bespeaks an undoubted staminal affinity of these petals. The second difference may be taken as significant of a foliar origin of the petals of *Asimina*, because the formation of a cortical system by the lateral traces is a characteristic sometimes displayed by leaves. So again a further study of the family will be interesting, especially a study of forms in which one whorl of petals is missing, reduced to mere scales, or is large and leathery.

The sepals differ from the sepals of the Ranunculaceae in that a

multitrace condition exists, and in that the traces have a tendency to pass cortically before entering the axial cylinder. Thus another question arises: Is this a characteristic of *Asimina triloba* alone or of the entire family? Only further investigations can answer this question.

An analysis of the data presented concerning the vascular anatomy of the flower of *Asimina triloba* reveals that it differs from the Ranunculaceae primarily in the development of a more or less definite cortical system of vascular strands, and in the foliar rather than staminal affinity of the petals. Because of these features a study of other members of the family is very desirable.

Summary

The important features of vascular anatomy of the flower of certain unusual forms of *Ranunculus*, *Caltha*, and *Delphinium*, together with the important features of floral vascular anatomy of the families Menispermaceae, Calycanthaceae, and Annonaceae have been presented. The facts pertinent to each of these groups have been discussed and summarized at the end of each section; but a general concluding summary of the features common to all four groups is necessary. The floral organization of these groups supports the view already expressed, that in the more primitive flower the carpels are three-trace and multiovulate; the stamens are single-trace structures, and the lowest may be normally and naturally in the form of petals, or all may be transformed into petals by horticultural manipulation; the sepals are three-trace trilacunar structures, but may become modified into single trace unilacunar or multitrace, multilacunar structures; all floral parts are spirally inserted and indefinite as to number. As regards the origin of the Menispermaceae, Calycanthaceae, and Annonaceae, the vascular skeleton of each gives undoubted evidence of origin from the Ranunculaceae. The study of the vascular skeleton of the flower of *Menispermum canadense* shows that it has been derived directly from some member of the Ranunculaceae which displayed a tendency toward cyclic arrangement of parts, the reduction of parts to a definite numerical plan, a uniovulate carpellary condition, and a single strand vascular supply to the individual appendage. At the same time the Menispermaceae

demonstrate that they do not lead to any advanced group. The definite development of the imperfect flower makes this impossible. The floral vascular skeleton of the Calycanthaceae demonstrates that this family is also a diverging side line from the Ranunculaceae. The receptacle has become cup-shaped, due to a checking of the growth of the apical (carpellate portion) floral primordium and a consequent bulging up of the surrounding floral primordium to form the cup. Such a condition illustrates a possible step in the mode of origin of the inferior ovary. However, the Calycanthaceae, as the Menispermaceae, are a closed line because the anatomical evidence at hand points to the inferior ovary of the Rosaceae and other dicotyledons as arising through a cohesion and adhesion of floral parts, rather than after the fashion suggested by *Calycanthus*. The floral vascular skeleton of the Annonaceae is clearly that of the Ranunculaceae, but possessing one peculiarity, a partially developed cortical vascular system. This characteristic is seldom found in a vegetative axis, and has never been found in another floral axis except possibly the Calycanthaceae; therefore the Annonaceae also represent a closed series. Thus the evidence gained from a study of these three families points to the Ranunculaceae as the crucial center of the order.

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BIOLOGICAL ABSTRACTS
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PA.

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FURTHER AGGLUTINATION TESTS WITH BACTERIAL PLANT PATHOGENS

I. BACTERIUM CAMPESTRE-BACT. PHASEOLI GROUP; BACT. MEDICAGINIS VAR. PHASEOLICOLA; BACT. TUMEFACIENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 378

GEO. K. K. LINK AND ADELINE DES. LINK[1]

Introduction

In recent papers, SHARP (7) and LINK and SHARP (5) have reported success in differentiating bacterial plant pathogens by the agglutination test. This test was applied by them to three yellow organisms which are not readily differentiated culturally, but which exhibit marked host specificity; namely, *Bacterium campestre* (a crucifer pathogen), *Bact. phaseoli* (a bean pathogen), and *Bact. phaseoli sojense* (a soy bean pathogen). It was also applied to a yellow organism, *Bact. flaccumfaciens*, which attacks the same host as *Bact. phaseoli*, but which causes quite distinct symptoms and also is culturally distinguishable from it. It was concluded that serological specificity is correlated with host specificity for *Bact. campestre*, *Bact. phaseoli*, and *Bact. phaseoli sojense*, and with symptom specificity for *Bact. phaseoli* and *Bact. flaccumfaciens*, and that the designation of these organisms as distinct species is justifiable serologically. It was also concluded that these organisms, while distinct, nevertheless are closely related.

The first part of the experiments reported here is a continuation and enlargement of the study of the yellow organisms of the so-called *Bact. campestre-Bact. phaseoli* group of SMITH (8). It was our plan to complete the agglutination studies of this group, which, in addition to *Bact. campestre* (black rot of crucifers), *Bact. phaseoli* (blight of beans), and *Bact. phaseoli sojense* (pustule of soy bean), might include *Bact. malvacearum* (angular leafspot of cotton),

[1] The Department of Hygiene and Bacteriology cooperated in providing space for the animals, and Dr. W. H. TALIAFERRO and Dr. L. G. TALIAFERRO of that department assisted us throughout the course of the experiments.

Bact. citri (citrus canker), *Bact. pruni* (blackspot and canker of plum and peach), *Bact. juglandis* (walnut blight), *Bact. translucens* (blight of barley), and possibly others. Of the latter we were able to obtain cultures of only *Bact. malvacearum*, *Bact. citri*, and *Bact. pruni*. We received, however, from Dr. E. F. SMITH cultures of *Bact. cucurbitae* (leafspot of squash), and also of an unnamed yellow organism which he asked us to test and which is here designated as *Bact. X*. Cultures of *Bact. citri*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. X* were not received until after immunization of the animals had begun, and as a result we were not able to make tests with their antisera.

The second part of the experiments here reported grew out of SHARP'S study (7) of bean pathogens. In addition to those tested by him, namely, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*, which are serologically closely related but distinct, we used *Bact. medicaginis* var. *phaseolicola*, a white organism which causes symptoms in the bean practically identical with those caused by *Bact. flaccumfaciens*, but which is culturally distinct from the latter and is closely related to *Bact. medicaginis* (2). This group of tests was made to determine whether *Bact. medicaginis* var. *phaseolicola* can be differentiated serologically from the other bean pathogens, and also to ascertain what serological relation, if any, it bears as a representative of the *Bact. medicaginis* group to the *Bact. campestre-Bact. phaseoli* group.

After the conclusion of the experimental work here reported, a paper of BROOKS, NAIN, and RHODES (1) which has bearing on this problem came to our attention. They applied the agglutination test to the following yellow organisms: *Bact. campestre*, *Bact. malvacearum*, *Bact. michiganense*, *Bact. pruni*, *Bact. phaseoli*, *Bact. phaseoli sojense*, *Bact. gummisudans*, *Bact. stewartii*, *Bact. pelargoni*, *Bact. vitians*, and *Bact. tracheiphilus*. The agglutinability of these organisms was tested in 1:100 dilutions of sera with titres of 1:8000. They concluded that a close relationship exists between *Bact. campestre* (certain strains), *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense*, *Bact. pelargoni*, and *Bact. vitians*. On the other hand, *Bact. michiganense*, *Bact. pruni*, *Bact. stewartii*, *Bact. tracheiphilus*, *Bact. hyacinthi*, and *Bact. lathyri* have no relationship to one another or to other members of the yellow group. One strain of

Bact. campestre stood by itself with regard to all the other organisms studied.

In order to put to an even more rigorous trial the suitability of the direct agglutination test as a means of differentiating and identifying bacterial plant pathogens, we included *Bact. tumefaciens* (crown gall pathogen), and tested it against the yellow organisms as well as against *Bact. medicaginis* var. *phaseolicola*. JENSEN (3), using the agglutination test, demonstrated that the American and Danish daisy strains of *Bact. tumefaciens* are distinct serologically. RIKER (6) reported success in developing in rabbits an antiserum of *Bact. tumefaciens*, with a titre of 1–3000.

In part II are reported agglutination experiments with members of the soft-rot group, *Bacillus aroideae* and *B. carotovorus*.

Sources of material

The cultures of *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* were of the same stock as those used by SHARP (7) and LINK and SHARP (5). From Dr. J. G. BROWN we obtained a culture of *Bact. malvacearum*, the pathogenicity of which was tested before the experiment. Cultures of *Bact. citri*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. X* were supplied by Dr. E. F. SMITH's laboratory; their pathogenicity was not tested by us. Dr. W. H. BURKHOLDER supplied us with a culture of *Bact. medicaginis* var. *phaseolicola* whose pathogenicity we did not test. The culture of *Bact. tumefaciens* came from isolations made from tomato galls produced by inoculation with a culture supplied us by Dr. A. J. RIKER in 1925.

Experimental procedure

Each organism was grown on standard potato-dextrose agar slants (pH 7.2) for four days. Even suspensions were then made of each organism in 50 cc. of 0.85 per cent NaCl solution. These suspensions were used as stock antigens for injections of rabbits and production of antisera. Two animals were used for each organism, so that the tests were run in duplicate. Each of the animals used was bled from the heart before the first injection, so that the serum might be tested for the presence of natural antibodies against all of the organisms to be tested later.

At first, following the method used by LINK and SHARP, we

injected suspensions of live organisms intravenously into the ear-vein of the animals. This method was successful with most of the animals but was fatal to some, especially to those injected with organisms of the soft-rot group. We tried intraperitoneal injections with these organisms, and found that they were less likely to be fatal to the animals. As a result, most of the antisera used in these experiments were obtained by injecting suspensions of live organisms intravenously five times into the ear-vein of the animals, at intervals of three days, using 0.5 cc. of the suspension for the first and 1 cc. for each of the remaining four injections; while a few of the antisera were obtained by intraperitoneal injections, giving 1 cc. of suspension of live organisms in the first injection and 2 cc. in each of two subsequent injections, with three-day intervals. The antisera produced by intraperitoneal injections are starred in the protocols. Seven days after the last injection the animals were bled from the heart, and the sera prepared and kept aseptically until they were used in the agglutination tests.

The cultures used for the preparation of suspensions in the agglutination tests were grown on standard beef extract-peptone-dextrose agar (pH 7.2). The organisms were washed off the agar with 0.85 per cent salt solution, and then shaken thoroughly in saline solution and centrifugated. The supernatant liquid was decanted, and the organisms were washed and centrifugated a second time. The last suspension in saline solution was used for the agglutination tests. The organisms were grown on different media, and washed in saline to take precaution against a positive precipitin reaction with the protein of the potato agar used in preparing the stock antigens.

Observations made in the course of tests with suspensions of the organisms against the normal sera led us to modify our procedure of making suspensions for the tests with the antisera. When we read the agglutination tests of the normal sera against suspensions in saline of *Bact. tumefaciens*, we found that there had been flocculation in all tests at all dilutions, but that most of the suspended organisms were still in suspension. The controls in saline behaved identically. Examination of the excess stock suspension which had been kept in the ice chest over night revealed that in this, too, there had been flocculation of a fraction of the organisms. Suspensions of *Bacillus*

TABLE I

TYPICAL PROTOCOL OF AGGLUTINATION TESTS

	TUBES										CONTROL
	1	2	3	4	5	6	7	8	9	10	
Antiserum { Dilution	1–5	1–10	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	.....
Antiserum { Amount in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
Bacterial suspension (antigen) in cc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Saline solution (0.85 per cent NaCl)	0	0	0	0	0	0	0	0	0	0	0.5
Final dilution of antiserum	1–10	1–20	1–40	1–80	1–160	1–320	1–640	1–1280	1–2560	1–5120	.....

carotovorus 3a and *B. aroideae* behaved similarly, but the settling was relatively slight, although it occurred in all tubes and in the saline control. Consequently, for the later tests of antisera against suspensions of *Bact. tumefaciens*, *B. carotovorus 3a*, and *B. aroideae* we separated the readily flocculating fraction by centrifugation at low speed, allowed the suspension to stand over night in the ice chest, and then pipetted off the supernatant suspension and used this in the agglutination tests. This method gave us suspensions which did not come down in the saline control. The possible significance of these findings has previously been discussed (4).

The sample protocol of table I shows the set-up used in each experiment. 0.2 cc. of antiserum was added to 0.8 cc. of saline solution (0.85 per cent NaCl), giving a dilution of 1–5. From this original dilution of 1–5 the other dilutions were obtained by mixing and then transferring 0.5 cc. of the diluted antiserum from tube 1 to tube 2, which contained 0.5 cc. of saline, from tube 2 to tube 3, and so on, up through tube 10 or 12. After thoroughly mixing the contents of each tube, 0.5 cc. of bacterial suspension in saline solution (antigen) was added to each tube, thus giving a dilution of 1–10 in tube 1, up through dilution 1–5120 in tube 10. The control in each set-up consisted of a tube containing 0.5 cc. saline and 0.5 cc. of bacterial suspension in saline. After thorough mixing by shaking, the series of tubes was incubated for one hour at 37° C. in a Wassermann bath, placed in a refrigerator for twelve hours, and then read both macroscopically and with a hand lens.

As a control for all series, a sample of serum drawn before immunization of each animal was tested against suspensions of the organisms to be used in tests against the antisera obtained from the animal. The set-up for these experiments was identical with that just described, save that normal serum (drawn before immunization) was used in place of antiserum.

The results of these experiments are presented in tables II to IX. The protocols of the tests for the presence of natural antibodies in the normal sera of the animals used are not given in detail. They are in each case identical with the protocols of the tests with the antisera; in other words, each protocol stands for two tests, one with the normal serum and the other with the antiserum of the animal against suspensions of the same organisms.

TABLE II*

ANTISERUM OF BACTERIUM CAMPESTRE AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre	87	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	−
	154	.....	+++	+++	+++	++++	++++	++++	++++	++++	++	−
Bact. citri	87	−	−	−	−	−	−	−	−	−	−	−
	154	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	87	−	−	−	−	−	−	−	−	−	−	−
	154	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	87	++	++	−	−	−	−	−	−	−	−	−
	154	−	−	−	−	−	−	−	−	−	−	−
Bact. pruni	87	−	−	−	−	−	−	−	−	−	−	−
	154	.....	−	−	−	−	−	−	−	−	−	−
Bact. X	87	++++	++++	++++	++++	+++	+++	+++	++	+	−	−
	154	.....	+++	+++	+++	+++	+++	+++	+++	++	+	−
Bact. medicaginis var. phaseolicola	87	−	+++	+++	+++	−	−	−	−	−	−	−
	154	.....	−	−	−	−	−	−	−	−	−	−
Bact. tumefaciens	87	+++	+++	+	−	−	−	−	−	−	−	−
	154	.....	−	−	−	−	−	−	−	−	−	−

* In table II and all the following tables where the symbols are used, ++++ stands for complete clearing of the suspension with total deposition in a clump; +++ stands for complete clearing but only partial deposition in a clump, many clumps being in suspension; ++ stands for partial clearing with a small clump and some clumps in suspension; + stands for partial clearing with a very small clump and a few clumps in suspension; and − stands for no change in turbidity. All readings were made macroscopically as well as with a hand lens.

In control tests with the sera of these animals before immunization, all tests were negative excepting those against suspensions of *Bact. tumefaciens*. These gave partial agglutination in all dilutions of sera as well as in the saline control (see page 181).

TABLE III*

ANTISERUM OF BACT. MALVACEARUM AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre	128†	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. citri	128	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	128	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	128	+++	−	−	−	−	−	−	−	−	−	−
	168	+++	++++	++++	++++	++++	++++	+++	++	−	−	−
Bact. phaseoli	128	−	−	−	−	−	−	−	−	−	−	−
	168	+++	+++	+++	+++	+++	+++	−	−	−	−	−
Bact. phaseoli sojense (S)	128	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. pruni	128	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. X	128	++	−	−	−	−	−	−	−	−	−	−
	168	++	++	++	−	−	−	−	−	−	−	−
Bact. flaccumfaciens	128	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. medicaginis var. phaseolicola	128	−	−	−	−	−	−	−	−	−	−	−
	168	−	−	−	−	−	−	−	−	−	−	−
Bact. tumefaciens	128	+++	−	−	−	−	−	−	−	−	−	−
	168	++	++	−	−	−	−	−	−	−	−	−

* For data of the control tests with sera of these animals before immunization, see footnote of table II.

† Injected intraperitoneally.

TABLE IV*

ANTISERUM OF BACT. PHASEOLI AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri	169	−	−	−	−	−	−	−	−	−	−	−
	170	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	169	−	−	−	−	−	−	−	−	−	−	−
	170	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	169	+++	+++	+++	+++	++	++	−	−	−	−	−
	170	+++	+++	+++	+++	+++	+++	++	−	−	−	−
Bact. phaseoli	169	++	++++	++++	++++	++++	++++	++++	++++	++++	+++	−
	170	+++	+++	++++	++++	++++	++++	++++	++++	++++	++	−
Bact. pruni	169	−	−	−	−	−	−	−	−	−	−	−
	170	−	−	−	−	−	−	−	−	−	−	−
Bact. X	169	++	++	++	+	+	−	−	−	−	−	−
	170	+++	++	++	++	++	−	−	−	−	−	−
Bact. medicaginis var. phaseolicola	169	++	++	++	++	−	−	−	−	−	−	−
	170	+++	+++	++	+	−	−	−	−	−	−	−
Bact. tumefaciens	169	+++	+++	++	+	+	−	−	−	−	−	−
	170	++++	+++	++	++	+	−	−	−	−	−	−

* For data of the control tests with sera of the animals before immunization, see footnote of table II.

TABLE V*

ANTISERUM OF BACTERIUM PHASEOLI SOJENSE (S) AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri	160†	−	−	−	−	−	−	−	−	−	−	−
	161	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	160	−	−	−	−	−	−	−	−	−	−	−
	161	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	160	−	++	++	++	−	−	−	−	−	−	−
	161	+++	+++	+++	+++	+++	+++	+++	++	++	−	−
Bact. phaseoli sojense (S)	160	++	++	+	+	+	+	+	−	−	−	−
	161	+++	+++	++	++	++	++	++	++	−	−	−
Bact. pruni	160	−	−	−	−	−	−	−	−	−	−	−
	161	−	−	−	−	−	−	−	−	−	−	−
Bact. X	160	+	+	+	+	−	−	−	−	−	−	−
	161	++	++	++	−	−	−	−	−	−	−	−
Bact. medicaginis var. phaseolicola	160	−	−	−	−	−	−	−	−	−	−	−
	161	++	++	++	++	−	−	−	−	−	−	−
Bact. tumefaciens	160	++	++	+	−	−	−	−	−	−	−	−
	161	+++	+++	++	−	−	−	−	−	−	−	−

* For data of the control tests with sera of the animals before immunization, see footnote of table II.

† Injected intraperitoneally.

TABLE VI*

ANTISERUM OF BACT. PHASEOLI SOJENSE (R) AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri	139†	−	−	−	−	−	−	−	−	−	−	−
	172	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	139	−	−	−	−	−	−	−	−	−	−	−
	172	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	139	++	++	+++	+++	+++	++	++	+	−	−	−
	172	++	++	+++	+++	+++	+++	+++	++	−	−	−
Bact. phaseoli sojense (R)	139	+++	+++	+++	+++	−	−	−	−	−	−	−
	172	++	++	++	−	−	−	−	−	−	−	−
Bact. pruni	139	−	−	−	−	−	−	−	−	−	−	−
	172	−	−	−	−	−	−	−	−	−	−	−
Bact. X	139	++	++	++	++	−	−	−	−	−	−	−
	172	++	++	++	+	+	−	−	−	−	−	−
Bact. medicaginis var. phaseolicola	139	++	++	++	−	−	−	−	−	−	−	−
	172	−	−	−	−	−	−	−	−	−	−	−
Bact. tumefaciens	139	+++	+++	+	−	−	−	−	−	−	−	−
	172	+++	++	+	+	+	+	−	−	−	−	−

* For data of the control tests with sera of the animals before immunization, see footnote of table II.
† Injected intraperitoneally.

TABLE VII*

ANTISERUM OF BACTERIUM FLACCUMFACIENS AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. citri	156	−	−	−	−	−	−	−	−	−	−	−
	157	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	156	−	−	−	−	−	−	−	−	−	−	−
	157	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	156	++	++	−	−	−	−	−	−	−	−	−
	157	++	−	−	−	−	−	−	−	−	−	−
Bact. pruni	156	−	−	−	−	−	−	−	−	−	−	−
	157	−	−	−	−	−	−	−	−	−	−	−
Bact. X	156	++	++	+	−	−	−	−	−	−	−	−
	157	−	−	−	−	−	−	−	−	−	−	−
Bact. flaccumfaciens	156	+++	++++	++++	++++	+++	+++	−	−	−	−	−
	157	+++	+++	+++	+++	+++	+++	++	−	−	−	−
Bact. medicaginis var. phaseolicola	156	+	+	+	+	−	−	−	−	−	−	−
	157	+	+	+	+	+	−	−	−	−	−	−
Bact. tumefaciens	156	+++	+++	−	−	−	−	−	−	−	−	−
	157	++	++	+	−	−	−	−	−	−	−	−

* For data of the control tests with sera of the animals before immunization, see footnote of table II.

TABLE VIII*

ANTISERUM OF BACT. MEDICAGINIS VAR. PHASEOLICOLA AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre	164†	−	−	−	−	−	−	−	−	−	−	−
Bact. citri	164	−	−	−	−	−	−	−	−	−	−	−
Bact. cucurbitae	164	−	−	−	−	−	−	−	−	−	−	−
Bact. malvacearum	164	−	−	−	−	−	−	−	−	−	−	−
Bact. phaseoli	164	−	−	−	−	−	−	−	−	−	−	−
Bact. phaseoli sojense (S)	164	−	−	−	−	−	−	−	−	−	−	−
Bact. pruni	164	−	−	−	−	−	−	−	−	−	−	−
Bact. X	164	−	−	−	−	−	−	−	−	−	−	−
Bact. flaccumfaciens	164	−	−	−	−	−	−	−	−	−	−	−
Bact. medicaginis var. phaseolicola	164	+++	+++	++++	++++	++++	++++	+++	−	−	−	−
Bact. tumefaciens	164	++	++	−	−	−	−	−	−	−	−	−

* For data of the control tests with sera of the animals before immunization, see footnote of table II.

† The second animal died after the last injection.

TABLE IX*

ANTISERUM OF BACT. TUMEFACIENS AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
Bact. campestre	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. citri	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. cucurbitae	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. malvacearum	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. phaseoli	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. phaseoli sojense (S)	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. pruni	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. X	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. flaccumfaciens	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. medicaginis var. phaseolicola	152	–	–	–	–	–	–	–	–	–	–	–
	153	–	–	–	–	–	–	–	–	–	–	–
Bact. tumefaciens	152	++++	++++	++++	++++	++++	++++	++++	++++	++	++	–
	153	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	–

* For data of the control tests with sera of the animals before immunization, see footnote of table II.

Discussion

In the tests of the normal sera, no agglutination was noted aside from that which occurred in all tubes which contained suspensions of *Bact. tumefaciens, B. carotovorus 3a,* or *B. aroideae.* That this agglutination was spontaneous and not due to the action of the normal sera is proved by the observations: (1) that it occurred equally in the control tubes containing only suspensions and saline; (2) that it did not occur in the saline controls in the second test in which suspensions were used from which the spontaneously agglutinating portion had been removed; and (3) that in the second test with the antisera of the same animals against the same organisms, no agglutination occurred in the higher dilutions in some instances.

In most cases three intraperitoneal injections (the first 1 cc. and the others 2 cc.) produced antisera with practically the same titres as five intravenous injections into the ear-vein (the first 0.5 cc. and the others 1 cc.). It is fortunate that antisera against plant pathogens can be produced by intraperitoneal injections, because this method is easier of manipulation and less likely to be fatal to animals than that of intravenous injection. Of the twenty-three animals injected intravenously eleven died, while of the eight injected intraperitoneally only two died. The only exception occurred with *Bact. malvacearum.* This organism when injected intravenously produced an antiserum which gave a titre of 1–1280 against suspensions of the homologous organism, but when injected intraperitoneally produced an antiserum which gave a titre of only 1–10 against its own suspensions (table III).

In the main the titres of the antisera of the two animals injected with the same organism are of the same magnitude. That there are idiosyncracies resident in individual animals is brought out in several cases. Thus (table II) the antiserum of *Bact. campestre* of one animal gave a titre against suspensions of *Bact. medicaginis* var. *phaseolicola, Bact. malvacearum,* and *Bact. tumefaciens,* while that of the other animal did not. In two other instances the antiserum of one animal reacted against suspensions of *Bact. medicaginis* var. *phaseolicola,* while that of the other did not (tables V and VI). Similarly, the antiserum of *Bact. flaccumfaciens* of one animal gave a titre against

suspensions of *Bact. X*, whereas that of the other animal failed to do so (table VII).

As in the experiments previously reported by LINK and SHARP, *Bact. campestre* again produced an antiserum which was highly specific for its homologous organism (table II). This antiserum reacted almost identically with suspensions of *Bact. X*. Although the titre obtained against this organism was not as high and the agglutination was slightly less in nearly all dilutions, this test indicates that these organisms contain antigens which lead to production of the same antibodies, and that they probably are closely related. Unfortunately we did not have any antiserum of *Bact. X* to test against suspensions of itself and of *Bact. campestre*, and consequently no final statement can be made as to their relationship. The antiserum of *Bact. campestre* gave either no titre, or a lower titre with suspensions of other yellow organisms which are taken to belong to the *Bact. campestre-Bact. phaseoli* group (*Bact. citri*, *Bact. malvacearum*, *Bact. pruni*, and possibly *Bact. cucurbitae*) than it did against suspensions of *Bact. medicaginis* var. *phaseolicola* and *Bact. tumefaciens*. The reaction against *Bact. malvacearum* was stronger than that against *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* (**5**, **7**).

The antiserum of *Bact. malvacearum* gave specific agglutination against suspensions of itself, good agglutination (titre 1–320) against *Bact. phaseoli*, and a slight agglutination against suspensions of *Bact. X*. It did not give reactions with suspensions of any of the other yellow organisms, nor with *Bact. medicaginis* var. *phaseolicola*, but did give a low titre against suspensions of *Bact. tumefaciens*.

Bact. phaseoli, which had produced an antiserum giving specific agglutination against suspensions of the homologous organism, and also agglutination of a lower titre against suspensions of *Bact. campestre* (**5**), *Bact. phaseoli sojense* (S), and of *Bact. flaccumfaciens* (**7**), again produced an antiserum which was specific against suspensions of itself (titre 1-5120), complete agglutination occurring in one case through dilution 1–5120, and in the other case through dilution 1–2560. A very good titre was also obtained against suspensions of *Bact. malvacearum* and a lower one with *Bact. X*. This seems to indicate that, serologically at least, *Bact. phaseoli*, *Bact. malva-*

cearum, and *Bact. X* are related. No titres were obtained against suspensions of the other yellow organisms. Titres of 1–80 and 1–160 were obtained respectively against suspensions of *Bact. medicaginis* var. *phaseolicola* and *Bact. tumefaciens*.

SHARP (7) and LINK and SHARP (5) found the antiserum of *Bact. phaseoli sojense* (S) to be specific when tested against suspensions of the homologous organism and of *Bact. campestre*, *Bact. phaseoli*, and *Bact. flaccumfaciens*. In our tests the antisera of *Bact. phaseoli sojense* (S) and (R) gave lower titres against suspensions of the homologous organisms than against suspensions of *Bact. malvacearum*. This may be interpreted as indicating that, in addition to the antigens which produce antibodies against *Bact. phaseoli sojense* (S) and (R), these organisms contain also antigens which stimulate the production of the same antibodies as *Bact. malvacearum*. Absorption of agglutinins might have enabled us to use the antisera of *Bact. phaseoli sojense* (S) and (R) to differentiate specifically between these organisms and *Bact. malvacearum*. The antisera of *Bact. phaseoli sojense* (S) and (R) gave agglutination in low dilutions against suspensions of *Bact. X*. Agglutination in low dilutions occurred also when these antisera were tested against suspensions of *Bact. medicaginis* var. *phaseolicola* and *Bact. tumefaciens*.

The antiserum of *Bact. flaccumfaciens*, according to SHARP (7) and LINK and SHARP (5), gives specific agglutination against suspensions of the homologous organism, and slight agglutination against suspensions of *Bact. campestre*, *Bact. phaseoli*, and *Bact. phaseoli sojense*. In these experiments its antiserum was highly specific for its homologous organism, giving a titre of 1–640, with complete agglutination through dilution 1–320. It gave slight agglutination against suspensions of *Bact. malvacearum* and of *Bact. X* (antiserum of one animal only). Very slight agglutination was obtained against suspensions of *Bact. medicaginis* var. *phaseolicola*, which causes practically identical symptoms in the bean. Stronger agglutination was obtained against suspensions of *Bact. tumefaciens* (titre 1–40, complete agglutination in dilution 1–20).

The antiserum of *Bact. medicaginis* var. *phaseolicola* was highly specific for its homologous organism, giving complete agglutination through the dilution range 1–10 to 1–640, but giving partial aggluti-

nation with suspensions of *Bact. tumefaciens* in low dilutions (1–10 to 1–20).

Bact. tumefaciens produced an antiserum which was extremely specific for its homologous organism, giving complete agglutination through the dilution range 1–10 to 1–5120 in one case, and 1–10 to 1–1280 in the other, and no agglutination at all with suspensions of all the other organisms tested.

On the basis of current interpretations of many immunologists, these results may indicate that while *Bact. campestre*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense* (S), *Bact. phaseoli sojense* (R), and *Bact. flaccumfaciens* contain some antigens (proteins) which stimulate the production of antibodies that react with *Bact. tumefaciens*, this organism does not contain any antigens (proteins) which stimulate production of antibodies that react with the other organisms. On this basis, therefore, *Bact. tumefaciens* can be differentiated specifically from the other organisms studied. Similarly, *Bact. medicaginis* var. *phaseolicola* can be differentiated from all the other organisms, including the bean pathogens *Bact. phaseoli* and *Bact. flaccumfaciens*, even though *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense* (S) and (R), and *Bact. flaccumfaciens* contain antigens which stimulate to a slight extent the production of antibodies that react with *Bact. medicaginis* var. *phaseolicola*. In other words, this organism, which in beans causes symptoms identical with those generally caused by *Bact. flaccumfaciens* and occasionally by *Bact. phaseoli*, is not only morphologically and physiologically distinct from these organisms, as determined by culture, but is also serologically distinct. In fact, serologically, *Bact. medicaginis* var. *phaseolicola* seems to stand closer to *Bact. campestre* and *Bact. phaseoli* than it does to *Bact. flaccumfaciens*.

Bact. malvacearum contains antigens that stimulate production of the same antibodies as *Bact. phaseoli*, and, to a less extent, as *Bact. X;* however, it contains in addition antigens that stimulate the production of antibodies that react only with its own suspensions. *Bact. malvacearum* can therefore be separated from the other yellow organisms on this basis. Serologically it seems to be more closely related to *Bact. phaseoli*, *Bact. phaseoli sojense* (S) and (R), and *Bact. X* than to *Bact. campestre* and *Bact. flaccumfaciens*.

The reaction of *Bact. X* to the antibodies of the *Bact. campestre* antigens is almost identical with that of *Bact. campestre*.

Bact. citri, *Bact. cucurbitae*, and *Bact. pruni* did not react with the antibodies produced by the antigens of any of the other organisms of the so-called yellow *Bact. campestre-Bact. phaseoli* group.

This raises the question whether the yellow organisms grouped by SMITH really constitute a single group. The results of SHARP (7) and LINK and SHARP (5) indicate that *Bact. campestre*, *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*, although distinct, are closely related serologically. The experiments here reported indicate that *Bact. malvacearum*, while distinct, also belongs to this group. This is in harmony with the findings of BROOKS, NAIN, and RHODES (1), who, however, found a closer relationship between *Bact. malvacearum* and *Bact. campestre* than we did. Although our data are not conclusive, because no tests were made with antisera of *Bact. citri*, *Bact. cucurbitae*, and *Bact. pruni*, they indicate strongly that these yellow organisms do not belong in the *Bact. campestre-Bact. phaseoli* group. BROOKS, NAIN, and RHODES had found by reciprocal tests that *Bact. pruni* does not belong to the *Bact. campestre-Bact. phaseoli* group.

Conclusions

1. The agglutination test can be used to differentiate *Bact. malvacearum* from the yellow organisms *Bact. campestre*, *Bact. phaseoli*, *Bact. citri*, *Bact. cucurbitae*, and *Bact. pruni*.

2. When the antiserum of *Bact. malvacearum* is tested against suspensions of *Bact. phaseoli sojense*, a specific differentiating test is obtained; when the antiserum of *Bact. phaseoli sojense* is used against suspensions of the homologous organism and of *Bact. malvacearum* the direct agglutination test is not specific and differentiating.

3. Serologically *Bact. malvacearum* is more closely related to *Bact. phaseoli* and *Bact. phaseoli sojense* than to *Bact. campestre*.

4. The yellow organisms tested probably are not a single group serologically. *Bact. campestre*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* apparently constitute a serological group.

5. *Bact. medicaginis* var. *phaseolicola* can be differentiated by the agglutination test from the bean pathogens *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*.

6. When the antiserum of *Bact. tumefaciens* is tested against suspensions of the homologous and of various heterologous organisms, highly specific agglutination occurs; when the antisera of these heterologous organisms are tested against suspensions of *Bact. tumefaciens*, agglutination generally takes place in the lower dilutions.

UNIVERSITY OF CHICAGO

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FURTHER AGGLUTINATION TESTS WITH BACTERIAL PLANT PATHOGENS

II. SOFT-ROT GROUP: BACILLUS AROIDEAE AND B. CAROTOVORUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 379[1]

GEO. K. K. LINK AND W. H. TALIAFERRO

Introduction

Several species of soft-rot bacteria have been described, but there has been no unanimity among investigators as to whether these are in fact distinct species. HARDING and MORSE (2) made extensive comparative studies of the soft-rot organisms, including *Bacillus aroideae*, *B. carotovorus*, *B. oleraceae*, and *B. omnivorous*, and thirty-nine so-called strains isolated from various hosts affected with soft-rot. They applied thirty-eight classificatory criteria, and concluded that the forty-three strains studied were alike except in their ability and manner of fermenting sugars. They placed *B. carotovorus*, *B. oleraceae*, *B. omnivorous*, and thirty of the unnamed strains into one group, on the basis of their forming acid and gas from dextrose, sucrose, and lactose; *B. aroideae* and three of the unnamed strains were put into a second group, on the basis of their forming acid but no gas from these sugars; the other six unnamed strains were considered intermediate between groups one and two. HARDING and MORSE felt that these differences were not sufficient to differentiate the organisms, and concluded that it was not advisable to consider *B. carotovorus* and *B. aroideae* distinct species. SMITH (5) considered *B. carotovorus*, *B. oleraceae*, and *B. omnivorous* apparently identical, and *B. aroideae* closely related to *B. carotovorus*. MASSEY (4), proceeding from the work of HARDING and MORSE and accepting their grouping of all soft-rot organisms as two species, *B. carotovorus* and *B. aroideae* (or preferably as one species, *B. carotovorus*), made a

[1] Joint contribution from this laboratory and from the Department of Hygiene and Bacteriology.

study of *B. carotovorus* and *B. aroideae*, using as criteria the hosts affected, and the action of these organisms on various organic carbon compounds. He found that calla, iris, kohl-rabi, and cauliflower can be used as hosts to differentiate *B. carotovorus* from *B. aroideae*. *B. carotovorus* gave positive results only when inoculated into iris, while *B. aroideae* did not rot iris but produced rot when inoculated into calla, kohl-rabi, and cauliflower. He verified the findings of HARDING and MORSE as to the action of these organisms upon sugars, and found further that they can be separated on the basis of their action upon ethyl alcohol media. He concluded that, although the organisms are closely related, they should be maintained as separate species. TAKIMATO (6) reported that serological tests indicate that the bacteria isolated from soft-rot of celery, lettuce, and radish belong to the same strain, although the radish organism differs from the others when grown in culture media.

We considered that it would be well to use the agglutination test to determine whether the host specificity reported by MASSEY for *B. aroideae* and *B. carotovorus* is correlated with serological specificity; that is, whether *B. aroideae* and *B. carotovorus* are serologically distinct. The experiments here reported consist of tests with *B. aroideae*, and with a strain of *B. carotovorus* here designated as *B. carotovorus* 3*a*. They also involve tests of these organisms against *Bact. campestre* of the *Bact. campestre-Bact. phaseoli* group, *Bact. medicaginis* var. *phaseolicola* of the *Bact. medicaginis* group, and *Bact. tumefaciens*.

After the conclusion of the experiments reported here, two papers with bearing upon this problem came to our attention. LACEY (3) made a study of the soft-rot organisms *B. carotovorus*, *B. solanisaprus*, and *B. phytophthorus*, applying cultural, pathological, and serological tests. These showed that there is a close relationship between these organisms, but that they are sufficiently different "to warrant their continued separation into distinct species." Even though there was common group agglutination, the species could be separated by the agglutination test. BERRIDGE (1) applied chemical agglutination tests to *B. solanisaprus*, *B. phytophthorus*, and *B. carotovorus*. She concluded that for the organisms of this group chemical agglutination tests are as reliable as serum agglutination tests. The chem-

ical agglutination tests show that *B. solanisaprus* and *B. phytophthorus* are not identical organisms, and are distinct from but closely related to *B. carotovorus*.

Source of cultures

A culture of *Bacillus carotovorus 3a* was obtained from Dr. J. G. LEECH, and a culture of *B. aroideae* was secured from Dr. A. B. MASSEY. We did not test the pathogenicity of these organisms.

Experimental procedure

The procedure was exactly that used in the experiments described in Part I; in fact, the first tests made on the soft-rot organisms were run at the same time as those reported in Part I, which should be consulted for the description of the experimental method and for the sample protocol. When the results of the first experiment with the soft-rot organisms were read, it was found that the dilutions had not been high enough to determine the titres in all cases; consequently some of each of the antisera was placed in an ice chest and saved for a test which was made a week later. In this, twelve instead of ten dilutions were used. It was found, however, that the additional dilutions were not necessary, because the titres of the antisera had gone down during the week's storage.

Discussion

The results of the experiments are recorded in tables I–V. The results of the tests with samples of sera drawn before immunization of each animal used against suspensions of the organisms used in the tests with the antisera later on are not presented in tabular form. The set-ups were exact duplicates of the tests with the antiserum of the same animal against suspensions of the organisms used.

As pointed out in Part I, some spontaneous agglutination or settling out occurred in tests of the normal sera of the animals against suspensions of *B. carotovorus 3a*, *B. aroideae*, and *Bact. tumefaciens*. This, however, also occurred in the control suspensions in saline. Later, proper precautions were taken in preparing suspensions, as indicated in Part I, and settling out was avoided. It was con-

TABLE I*

ANTISERUM OF BACTERIUM CAMPESTRE AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
B. aroideae.....	87	++	++	−	−	−	−	−	−	−	−	−
	154	.....	++	+	−	−	−	−	−	−	−	−
B. carotovorus 3a...	87	−	−	−	−	−	−	−	−	−	−	−
	154	.....	−	−	−	−	−	−	−	−	−	−
Bact. campestre....	87	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	−
	154	+++	+++	+++	++++	++++	++++	++++	++++	++++	++	−

* In table I and all the following tables where the symbols are used, ++++ stands for complete clearing of the suspension with total deposition in a clump; +++ stands for complete clearing but only partial deposition in a clump, many clumps being in suspension; ++ stands for partial clearing with a small clump and some clumps in suspension; + stands for partial clearing with a very small clump and a few clumps in suspension; and − stands for no change in turbidity. All readings were made macroscopically as well as with a hand lens.

In control tests with the sera of these animals before immunization, all tests were negative excepting those against suspensions of *B. carotovorus 3a* and *B. aroideae;* these gave very slight agglutination or settling in all dilutions of sera, as well as in the saline control (see page 200).

TABLE II*

ANTISERUM OF BACTERIUM MEDICAGINIS VAR. PHASEOLICOLA AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
B. aroideae.....	164†	—	—	—	—	—	—	—	—	—	—	—
B. carotovorus 3a...	164	—	—	—	—	—	—	—	—	—	—	—
Bact. medicaginis var phaseolicola.....	164	+++	+++	++++	++++	++++	++++	+++	—	—	—	—

* For data of the control tests with sera of the animals before immunization see footnote for table I.
† The second animal died after the last injection.

TABLE III*

ANTISERUM OF BACTERIUM TUMEFACIENS AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TUBES										CONTROL
		1	2	3	4	5	6	7	8	9	10	
B. aroideae.....	152	++	++	++	—	—	—	—	—	—	—	—
	153	++	++	++	++	—	—	—	—	—	—	—
B. carotovorus 3a...	152	—	—	—	—	—	—	—	—	—	—	—
	153	—	—	—	—	—	—	—	—	—	—	—
Bact. tumefaciens...	152	++++	++++	++++	++++	++++	++++	++++	++++	++	++	—
	153	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	—

* In control tests with the sera of these animals before immunization, all tests gave very slight agglutination or settling in all dilutions of sera as well as in the saline control (see page 200).

cluded from the results that the sera of none of the animals before immunization agglutinated suspensions of *B. carotovorus* 3*a* or *B. aroideae*.

The antisera of *Bact. campestre*, *Bact. medicaginis* var. *phaseolicola*, and of *Bact. tumefaciens* failed to give agglutination in any dilutions (1–10 to 1–5120) with suspensions of *B. carotovorus* 3*a*. The antiserum of *Bact. medicaginis* var. *phaseolicola* also failed to give agglutination in any dilution with suspensions of *B. aroideae*. The antisera of *Bact. campestre* gave partial agglutination in dilutions 1–40 with suspensions of *B. aroideae*, and the antisera of *Bact. tumefaciens* gave partial agglutination in dilutions 1–80 against suspensions of *B. aroideae*.

The antisera of *B. aroideae* and of *B. carotovorus* 3*a* gave no agglutination against suspensions of *Bact. campestre* and *Bact. medicaginis* var. *phaseolicola*, but did give titres of 1–320 against suspensions of *Bact. tumefaciens*. Obviously, then, the agglutination test can be used to differentiate the soft-rot organisms *B. aroideae* and *B. carotovorus* from *Bact. campestre*, *Bact. medicaginis* var. *phaseolicola*, and *Bact. tumefaciens*.

The antiserum of *B. aroideae* (animal no. 173) tested against suspensions of the homologous organism gave complete agglutination through all dilutions (1–10 to 1–5120) in one test, and a titre of 1–10240 with complete agglutination in dilutions 1–10 to 1–1280 in the second test. The antiserum obtained from the second animal (no. 174) gave complete agglutination in all dilutions in the first test (1–10 to 1–5120), and a titre of 1–20480 with complete agglutination in dilutions 1–10 to 1–1280 in the second test. In addition, the antisera of *B. aroideae* also gave group agglutination against suspensions of *B. carotovorus* 3*a*. The antiserum (animal no. 173) tested against suspensions of *B. carotovorus* 3*a* gave a titre of 1-5120 with only partial agglutination in all dilutions in the first test, and a titre of only 1–160 in the second test; the other antiserum (animal no. 174) in the first test gave a titre of 1–80 with complete agglutination in dilutions 1–10 to 1–40, and in the second test gave the same titre but complete agglutination only in dilution 1–10. Apparently the antiserum of *B. aroideae* can be used to diagnose *B. aroideae*, and to differentiate *B. aroideae* from *B. cartovorus* 3*a*.

TABLE IV*

ANTISERUM OF B. AROIDEAE AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TEST	TUBES 1	2	3	4	5	6	7	8	9	10	11	12	CONTROL
B. aroideae	173†	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	.....	.....	−
		2	+++	++++	++++	++++	++++	+++	+++	+++	++	+	+	−	
	174	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	.....	.....	−
		2	++++	++++	++++	++++	++++	++++	+++	+++	++	++	++	++	
B. carotovorus 3a	173	1	++	++	++	++	+	+	+	+	+	+	.....	.....	−
		2	++	++	+	+	+	−	−	−	−	−	−	−	
	174	1	+++	+++	+++	+	−	−	−	−	−	−	.....	.....	−
		2	+++	++	++	++	−	−	−	−	−	−	−	−	
Bact. campestre	173	.....	−	−	−	−	−	−	−	−	−	−	.....	.....	−
	174	.....	−	−	−	−	−	−	−	−	−	−	.....	.....	
Bact. medicaginis var. phaseolicola	173	.....	−	−	−	−	−	−	−	−	−	−	.....	.....	−
	174	.....	−	−	−	−	−	−	−	−	−	−	.....	.....	
Bact. tumefaciens	173	.....	++	++	+	−	−	−	−	−	−	−	.....	.....	−
	174	.....	+++	+++	++	+	+	+	−	−	−	−	.....	.....	

* In control tests with sera of these animals before immunization, the tests with *Bact. campestre* and *Bact. medicaginis* var. *phaseolicola* were negative; the tests with *B. aroideae*, *B. carotovorus* 3a and *Bact. tumefaciens* gave slight agglutination or settling in all dilutions of sera as well as in the saline control (see page 200).

† Injected intraperitoneally.

TABLE V*

ANTISERUM OF B. CAROTOVORUS 3A AGAINST

SUSPENSION OF (ANTIGEN)	ANIMAL	TEST	TUBES												CONTROL
			1	2	3	4	5	6	7	8	9	10	11	12	
B. aroideae	66†	1	++++	++++	++	++	++	++	+	−	−	−	.....	.....	−
		2	+++	+++	++	+	+	+	−	−	−	−	−	−	−
B. carotovorus 3a	66	1	++++	++++	++++	++++	++	++	++	++	−	−	.....	.....	−
		2	++++	++++	++++	++++	++	++	++	++	+	−	−	−	−
Bact. campestre	66	.....	−	−	−	−	−	−	−	−	−	−	.....	.....	−
Bact. medicaginis var. phaseolicola	66	.....	−	−	−	−	−	−	−	−	−	−	.....	.....	−
Bact. tumefaciens	66	.....	++++	++++	+++	++	++	++	−	−	−	−	.....	.....	−

* In control tests with sera of these animals before immunization, the tests with *Bact. campestre* and *Bact. medicaginis* var. *phaseolicola* were negative; the tests with *B. aroideae*, *B. carotovorus*, and *Bact. tumefaciens* gave slight agglutination or settling out in all dilutions of sera as well as in the saline control (see page 200).

† Injected intraperitoneally.

The antiserum of *B. carotovorus* 3*a* tested against suspensions of the homologous organism gave titres of 1–1280 and 1–2560 with complete agglutination in dilutions 1–10 to 1–80 (++++) in both sets of tests. It also gave group agglutination with suspensions of *B. aroideae*. Against suspensions of this organism it gave titres of 1–640 and 1–320 with complete agglutination (++++) in dilutions 1–10 to 1–20 in the first test, and in dilutions 1–10 and 1–20 in the second test. These tests indicate that the antiserum of *B. carotovorus* 3*a* lends itself to differentiation between *B. carotovorus* 3*a* and *B. aroideae*.

These results can be interpreted to indicate that, although *B. aroideae* contains some of the antigens which stimulate production of the same antibodies as *B. carotovorus* 3*a*, it also contains others that stimulate production of different antibodies from *B. carotovorus* 3*a*; therefore *B. aroideae* can be differentiated from *B. carotovorus* 3*a* on this basis. The results can be interpreted to indicate further that, while *B. carotovorus* 3*a* contains antigens which stimulate production of antibodies that react with suspensions of *B. aroideae*, it apparently contains others to some extent that stimulate production of different antibodies from *B. aroideae;* therefore it can be differentiated from *B. aroideae* on this basis.

Conclusions

1. The agglutination test can be used to differentiate *Bacillus aroideae* and *B. carotovorus* of the soft-rot group from *Bacterium campestre* of the *Bact. campestre-Bact. phaseoli* group, from *Bact. medicaginis* var. *phaseolicola*, and from *Bact. tumefaciens*.

2. Serologically, *B. aroideae* and *B. carotovorus* are distinct although closely related.

3. Maintenance of *B. aroideae* and *B. carotovorus* as distinct species is justified serologically.

UNIVERSITY OF CHICAGO

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ABSCISSION IN COTTON FLOWERS[1]

CHARLES P. DUTT

(WITH SEVEN FIGURES)

The cotton plant is sensitive to sudden changes in its environment, and this sensitiveness is manifested by modified growth, coloration of the bark and leaf, and the shedding of leaves and flowers. There may also be other manifestations. Abscission has been described as self-pruning of unnecessary parts of the plant, but considered from the growers' point of view, any practice by which abscission in the cotton plant could be prevented or minimized and by which the bolls could be brought to maturity would be an advantage. The causes in detail and the mechanism of abscission are little understood. Some work has been done to determine the exact changes that take place, but there is not sufficient evidence for some of the explanations that have been advanced.

Since there is some non-uniformity in the meaning of terms used by various investigators who have worked with abscission, the terms used in this paper (which are those upon which the majority of investigators agree) are briefly defined as follows: Abscission is the detachment of an organ by the separation of actively living cells. The separation layer is the layer of cells, the components of which separate at time of abscission. The separation cells or abscis cells are cells that make up the separation layer. The abscission zone is the general region in which abscission occurs.

Historical review

VON MOHL (17) was the first to announce that a definite separation layer is formed at the base of the petiole before the fall of the leaf. He pointed out that abscission always occurs by the separation of the cells from one another while their walls are still intact. WIESNER (21) confirmed this observation, and formulated the theory that the dissolution of the intercellular substance of the cells of the sep-

[1] Contribution from Division of Agronomy, University of California.

aration layer is caused by the development of organic acid in the leaves. MANGIN (16) discovered the pectic nature of the middle lamella of cell walls in plants, and since this discovery abscission has been referred to as a dissolution of the middle lamella.

The anatomical workers disagree as to the amount of cell wall altered during abscission. LEE (10) reported the disappearance of the middle lamella in foliar abscission. HANNIG (6) reported the same condition in the abscission of flowers of *Mirabilis jalapa*. TISSON (20) and LEE (10) reported that the secondary layer of the cell wall also was altered to some extent, only the thin tertiary membrane lining the cell cavity remaining. LLOYD (12) found the same condition in the abscission of cotton bolls and flowers of *Mirabilis jalapa*. SAMPSON (19) and HODGSON (7) suggested that enzymic activity may be an important factor in abscission.

BALLS (2) reported that the abscission zone of cotton flowers is marked by an external groove. He described the abscission layer as a layer of meristematic cells, one cell thick. The divisions begin in the cortex and extend inward; the dividing wall between the daughter cells splits immediately along its middle lamella. The daughter cells which are left on the face of the scars, after the pedicel has been broken away, bulge outward and form a callus. LLOYD (15) found that in young cotton bolls only one tier of cells is involved, while in old tissues, such as the internode, there are ten to twelve tiers of cells involved, and these show evidence of physiological activity. He stated that the greatest physiological activity occurs in one or two tiers of cells which occupy the upper limit of the abscission zone and constitute the separation layer. The cell walls are altered most in the separation layer. This alteration proceeds so far as to digest completely a part of the primary and secondary walls.

ATKINSON (1) held the view that abscission is a "purely physiological trouble." He stated that the immediate cause in cotton flowers is the interference of a saturated soil with root activity. BALLS (2) stated that abscission is due largely to environmental influence and not exclusively to soil water conditions, although the position of the water table has been shown to have a major influence in the irrigated regions of Egypt. He induced boll abscission by asphyxiating the root system, by immersion in water, and by root

pruning. BALLS found a rapid loss of water from bolls when removed from the plant, and suggested that there might be an actual net loss of water during the day if the roots were unable to supply the amount needed. A rising water table may reduce the roots, and may produce the same condition. The work of LIVINGSTON and BROWN (11), and of LLOYD (14) on the deficit of water in leaves, and BALLS' own observations on the cessation of growth in sunshine favor this view. BARANETSKY (3), working on the daily periodicity of growth, found that plants grow more slowly by day than by night; so it appears that light, as well as a deficit of water, exerts a retarding influence upon growth.

BALLS noted that wilting, or even a water deficit sufficient to lead to the closure of stomata, would result in the raising of the internal temperature, which might serve as a stimulus to abscission, especially when the change is sudden. He states: "we are unable to form a clear conception of the chain which extends from the severed root to the absciss layer." He is almost certain, however, that the main factor, if not the only one, is the water content of the plant. The absciss layer is the name given by BALLS to the separation layer.

BALLS, and LLOYD (15), found the transpiration rate of cotton leaves to be very high. BALLS found a loss of 1.8 gm. per 100 sq. cm. per hour, and LLOYD found the highest loss to be 5.25 gm. per 100 sq. cm. per hour. This indicates a heavy draft upon the water supply of the plant. LLOYD found that the water deficit limited growth; that the depletion of soil moisture was the cause of the gradually increased shedding as the season progressed; and that such shedding could be offset by irrigation.

The evidence obtained on the cotton plant by BALLS in Egypt and LLOYD in America strongly indicates that lack of water constitutes a stimulus leading to floral abscission. The work of PRESCOTT (18), on the other hand, shows that heavy irrigation may cause increased shedding. He found that when plants were given excessive amounts of water all the flowers were shed. As the amount of water was reduced the shedding was also decreased. Under "heavy," "medium," and "light" irrigations 85.4, 60.8, and 59.0 per cent of the flowers were shed respectively. With very light irrigation the shedding was 57.3 per cent. When a small amount of water was applied,

not only was the shedding reduced but the total number of flowers per plant was also greatly reduced. PRESCOTT's work does not harmonize with LLOYD's theory that shedding may be offset by irrigation.

HAMMOND (5) reported a very high rate of shedding under normal field conditions. The data are reproduced in table I, the monthly totals being summarized from the daily counts.

LLOYD (15) found that the time required for abscission varies with the age of the flowers and bolls. He found that squares and

TABLE I

NORMAL SHEDDING IN COTTON

	DATE OF EMERGENCE			
	April	May	June	July
Number of plants	7	10	2	1
Forms appeared	1700	2586	154	60
Forms died	1231	1819	106	24
Forms bloomed	469	667	48	36
Blooms and bolls dropping	163	199	12	19
Bolls maturing	306	468	36	7
Per cent maturing	18	18	23	11

flower buds were the most susceptible to shedding. As the bolls become older they are less likely to be abscissed. The bolls do not shed after the tenth day, unless wounded by insects or mechanical means. This investigator found that shedding was very rapid in young squares or unopened flowers. The abscission may occur before or after flowering, but during anthesis there occurs a partial or complete inhibition of the abscission process. He found the usual period for abscission in squares to be 48 hours. LLOYD was able to cause 100 per cent abscission of flowers by preventing pollination. He found the abscission period to be extended with the increasing age of the bolls, the maximum length of time being 6 days or less.

LLOYD (15) found that prevention of pollination by the removal of stamens was followed by total shedding of the young bolls in 48 hours. HODGSON, working with LLOYD and JIVANNA RAO (8), found that if pollen grains were placed in water, they burst immediately and were rendered impotent for fertilization. LLOYD points out that

rain on open flowers undoubtedly causes the pollen grains to burst, or otherwise injures the flower, and that the damage is lessened where the flowers are protected by overhanging leaves. The time of rainfall is undoubtedly very important; a light shower when the pollen is being discharged might do more damage than a heavy rain at any other time.

KENDALL (9), working on abscission of fruits and flowers in the Solanaceae, was unable to induce abscission with narcotic vapors, injury to floral organs, sudden rise in temperature, lack of pollination, or changes in soil conditions. The last he recognizes as an indirect cause.

GOODSPEED and KENDALL (4) report that more cells are involved in abscission in the F_1 generation of *Nicotiana* hybrids made with *Nicotiana sylvestris* than in the parents. This indicates that abscission is involved in Mendelian heredity.

Technique

The writer has studied the abscission zone and separation layer in pedicels of cotton. Microscopic examination of fresh freehand sections was supplemented with preparations made by the paraffin method. The freehand sections were mounted in water without killing. The microchemical tests were made on somewhat thick freehand sections; thin sections were found to be unsatisfactory. The material used for sectioning in paraffin was killed in formalin-alcohol, absolute alcohol, and various concentrations of the chromo-acetic series. Weak chromo-acetic solution (Shaffner's formula) gave best results.

Longitudinal sections were supplemented by cross-sections through the base of the pedicel. The sections were cut 10 μ in thickness, and stained with safranin and Delafield's haematoxylin.

Abscission types

The approximate position of abscission is usually marked by a faint external groove (figs. 1–3). This groove is present in mature flower bud pedicels, but is faint in very young ones. The groove is formed as a result of the difference in the rate of growth of the corti-

cal cells. In general there are two types of grooves; it may be ovate or somewhat rounded, extending about the same distance above and below the point of attachment (fig. 1), or it may be ovate, extending toward the lower node and having a considerable extension on the lower side. In extreme cases it may extend the entire length of the internode, and result in the removal of a strip of cortex (fig 3).

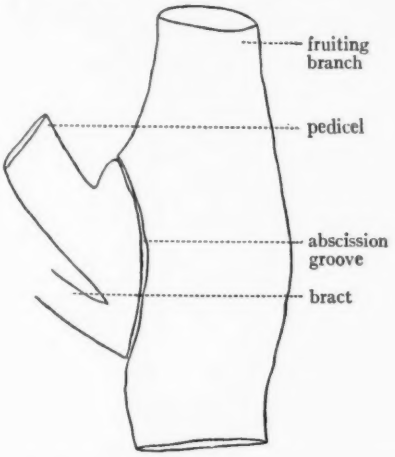

FIG. 1

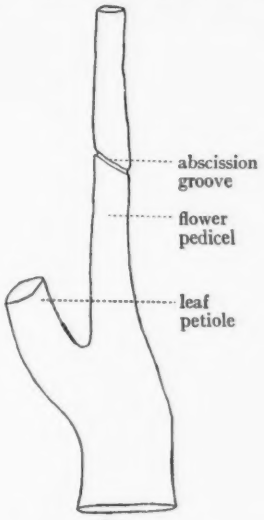

FIG. 2

FIGS. 1, 2.—Fig. 1, side view of circular abscission. Fig. 2, type of abscission in which only portion of pedicel is abscissed.

Observations show that when the groove is ovate or somewhat rounded abscission is complete (figs. 4, 6). In this type the groove is continuous through pedicel and petiole. The position of the groove is in the internode at the base of the pedicel, slightly back of the juncture of the leaf petiole. When the groove occurs near the center of the internode, the leaf petiole is not involved (fig. 2). In this type the abscission is complete, the abscissed part falls off very readily unless it is old, and the vascular tissues have become well developed. When the groove extends down the stem for a considerable distance the excised part often persists and dies in place, drying slowly. In attached ones there remains a slight connection of the vascular system, which prevents rapid drying.

Abscission zone

The external groove indicates in a general way the position of the abscission zone, although the latter does not always cross in the plane indicated by the external groove. It may develop to either side of it or along its upper edge, but in the majority of cases the abscission zone coincides and lies directly beneath it. When young pedicels are examined there is no apparent differentiation of any anatomical structure, hence the abscission zone does not appear to be pre-formed.

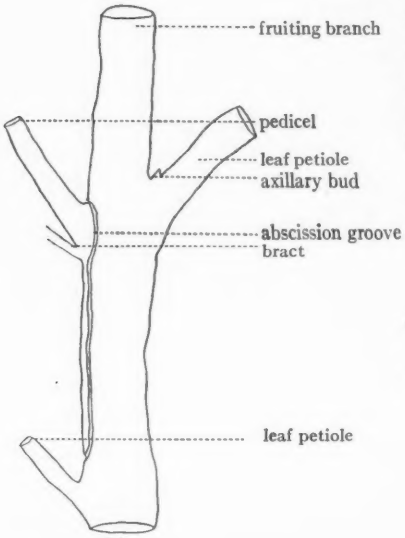

FIG. 3

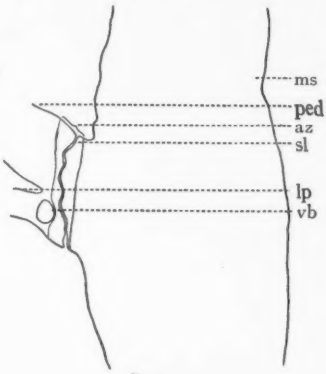

FIG. 4

FIGS. 3, 4.—Fig. 3, side view of downward projecting abscission. Fig. 4, diagram showing various parts of abscission: *ms*, main stem; *ped*, flower pedicel; *az*, abscission zone; *sl*, separation layer; *lp*, leaf petiole; *vb*, vascular bundle (note that abscission zone and separation layer are deflected from vascular bundle).

The abscission zone involves ten to twelve tiers of cells (fig. 5). The cell walls of the abscission zone swell and may become twice the normal thickness (fig. 6), and their power to take up and hold stains is reduced.

Contrary to the findings of BALLS, there is no evidence of cell division in the abscission zone at any time during the process.

Actual separation usually involves one or two rows of cells (fig.

6). The greatest physiological activity occurs in this layer, the tissue disintegrating by the dissolution of the cell walls. Separation does not occur straight across the pedicel; in most cases it follows a zigzag

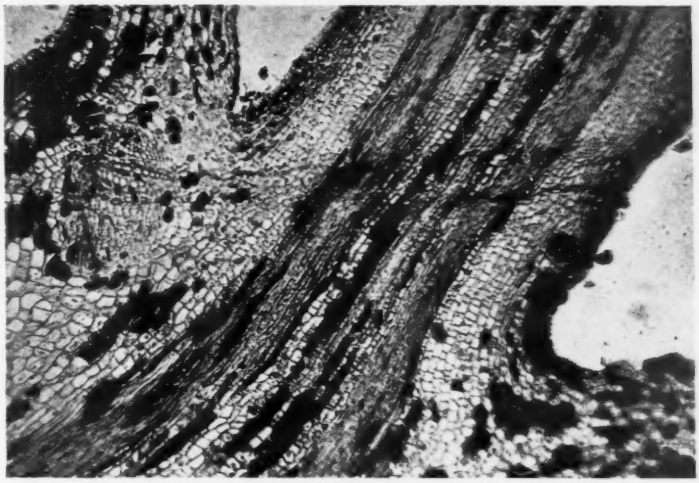

FIG. 5.—Early stage of abscission zone; petiole at upper left, pedicel at upper right

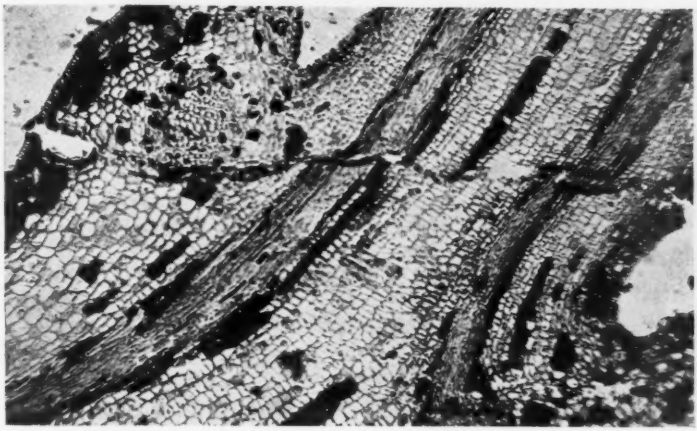

FIG. 6.—Separation layer with pockets; petiole at upper left, pedicel at upper right

course (fig. 4) and often contains pockets (fig. 6). These pockets contain single cells or groups of cells that have resisted chemical alteration.

Abscission process

Before abscission is initiated the cells of the abscission zone are well supplied with starch. The starch here is far in excess of the amount present in the tissues on either side. This relationship is reversed as soon as abscission begins, that is, the starch is then more abundant in the surrounding tissues than in the abscission zone. By the time abscission starts, a large amount of the starch is either used up or removed by translocation, although some of it remains until the separation layer becomes well defined. The rapid chemical changes which occur in the cell walls make doubtful the removal of starch from the cells by translocation. The starch is most likely used by these cells as a source of energy.

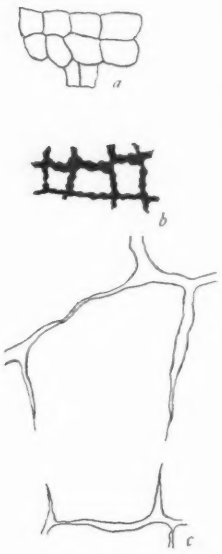

FIG. 7.—Cells from separation layer showing changes in cell walls: *a*, normal cells; *b*, thickened cell walls; *c*, cell showing dissolution of wall.

If thick longitudinal sections through the abscission zone are stained with chloriodide of zinc and potassium iodide-iodine a purple band is formed, delimiting the abscission zone. Examination of sections at subsequent stages shows that the starch content in the cells of the abscission zone decreases as abscission progresses.

The second indication of a change in the abscission zone is the thickening of the cell walls, which become about twice the original thickness (fig. 6).

The earliest indication of chemical alteration is the decreased power of the cell wall to take and hold stains. Thick sections stained with iodine previous to abscission show the cell walls distinctly colored, but if stained shortly after the beginning of abscission the cell walls show a much lighter color than adjacent cells outside of the

abscission zone. This indicates a chemical change of cellulose to hydrocellulose.

If thick sections are boiled in weak hydrochloric acid (about 4 per cent) for one minute and then treated with strong potassium iodide-iodine, the cell walls of the abscission zone show a faint greenish hue. If the sections are washed gradually, the yellow of the iodine disappears and the walls show a pale blue, indicating hydrocellulose. This pale blue fades away if washing is continued, but the color does not fade in the cells adjacent to the abscission zone. The fading of the stain shows the decreased power in holding it, since the color intensifies as the distance from the abscission zone increases. Thick sections, stained in dilute methylene blue overnight, show that the cells of the abscission zone do not hold the stain as do the neighboring cells. The decreased power of the cell walls to take up and hold stains indicates a chemical alteration in them.

Microchemical examination shows that the cell walls are composed of cellulose before abscission sets in. If sections are treated with 75 per cent sulphuric acid, then stained with iodine, a blue color develops in the cell walls, indicating cellulose. If sections are stained with methylene blue after the process of abscission is under way, a violet color develops in the center of the cell wall, indicating the presence of pectic substances.

If thick sections are treated with 5 per cent sulphuric acid, large needle crystals of calcium sulphate are formed in the cells. These tests show calcium to be present in all cells before abscission, but as abscission nears completion it decreases. When abscission is completed the calcium disappears in the abscission cells, while it is plentiful in adjacent cells.

A partial chemical alteration involves a zone of ten to twelve rows of cells (fig. 5). The greatest degree of alteration takes place in one or two rows of cells near the boll side of the abscission zone. The cell walls of these rows of cells are completely altered and dissolved. This produces a complete separation (fig. 6), but does not necessarily occur directly across the pedicel. The separation layer first forms in the innermost layer of the cortex, and is propagated outward toward the epidermis. The abscission zone forms in the pith, separately but simultaneously. The abscission is completely

inhibited in the tracheae of the vascular system. The tracheal tubes show evidence of being pulled apart mechanically.

Relation of soil moisture to water content of cotton

Study of the abscission problem raised the question of the relation of the moisture content of the soil to the moisture content of different parts of the cotton plant, consequently samples of different parts of the plant were taken on four different dates, and at the

TABLE II

MOISTURE CONTENT OF COTTON TISSUES AND SOIL

	DATE OF SAMPLE			
	August 19	August 23	August 31	September 6
	Percentage moisture content of tissues			
Flower	82.86	82.22	81.27	80.22
Young bolls	85.79	85.22	85.34	82.22
Mature bolls	85.62	81.61	82.73	80.20
Upper leaves	76.31	74.06	73.17	73.08
Lower leaves	75.15	74.90	73.84	72.75
Upper stem	83.52	71.86	82.10	68.48
Middle stem	75.43	71.58	70.51	65.63
Lower stem	70.98	65.87	67.05	64.01
Root	67.95	69.12	71.99	68.31
Pedicel normal	82.49	81.01	78.09	77.05
Pedicel abscissing	74.06	73.09	67.02	64.46
	Percentage moisture content of soil			
First foot	20.33	15.65	13.61	11.38
Second foot	20.65	15.40	15.00	12.55
Third foot	20.12	19.53	13.66	11.84
Fourth foot	19.32	18.13	16.63	11.58
Average of 4 feet	20.10	17.17	14.72	11.84

same time soil samples were taken under the plant sampled. All samples were in duplicate and oven dried at 100° C. The soil was irrigated on August 18. The results are shown in table II.

The figures in the table bring out one striking relationship, namely, that in general the water content of the cotton plant decreases as the water content of the soil decreases, showing the direct effect of soil moisture on the water content of the cotton plant. It should be noted that young tissue, such as flowers, leaves, and young bolls decline only slightly in moisture content as time from irrigation in-

creases; the moisture in roots even increased over the three-week period. The moisture content of both the normal and abscissing pedicel declined rapidly.

Summary

1. Abscission of the cotton flower usually occurs at or near the base of the internode, but sometimes in the middle.

2. A groove or surface depression generally indicates the position of the abscission zone.

3. In very young material there are no apparent histological differences setting off the abscission zone from the tissues on either side.

4. The abscission zone consists of ten to twelve rows of cells.

5. The separation layer is located near the upper or distal region of the abscission zone, and consists of one or two rows of cells.

6. The separation layer is formed by the chemical dissolution of the cell walls.

7. There is no evidence of cell division in the abscission zone of the cotton flower.

8. All the tissues across the pedicel except the tracheal tubes function in abscission.

9. The first visible indication of abscission is the swelling of the cell walls, followed by their partial solution.

10. Prior to abscission, large quantities of starch are stored in the abscission zone, which later disappears during the development of the abscission zone.

UNIVERSITY FARM
DAVIS, CALIF.

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STUDIES IN THE GENUS MENTHA

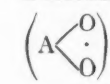
15. ACTION OF PEPPERMINT PEROXIDASE ON MENTHOL

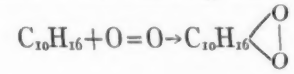
SAMUEL M. GORDON[1]

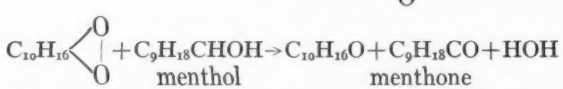
In a previous paper (6) on the quantitative relations of catalase and peroxidase in the growing peppermint plant, it was shown that both of these reached a maximum at the flowering period. Just past the flowering period these enzymes took a decided drop. It was further shown that the florets showed little oxidizing capacity in terms of catalase and peroxidase values. Whether the increase in the oxidizing enzymes in the leaf at the flowering period is related to the oxidation of menthol is difficult to decide, in view of the contradictory analytical values of CHARABOT (3) and KLEBER (7). On the one hand, CHARABOT claimed that the content of menthone (oxidation product of menthol) increased with the vegetative process, and at the expense of the previously formed menthol. KLEBER, however, showed that the oil derived from younger plants was richer in menthone. The former worker also claimed that when the plants were deprived of their inflorescences the oxidation of menthol was insignificant. The results of CHARABOT are harmonized with the oxidase values by assuming a transportation of the menthone from the leaf to the floret. It has not definitely been ascertained, however, that the plant or an extract of it can bring about this oxidation. If an extract or some portion of the plant were available, which could oxidize the alcohol to the ketone, such an assumption could safely be assumed. BROOKS (2) attempted to carry out such an oxidation but obtained a negative result. The extract used by him yielded the usual color test for oxidases.

Once more taking CHARABOT's analytical results as a point of departure, it will be recalled that his data showed an increase in the amount of unsaturated hydrocarbons present at the period of inflorescence, and he states that at this time an increased oxygen absorption is taking place. This increased content of hydrocarbon takes on

[1] National Research Council Fellow in Biological Sciences.

a new importance in the light of the autoxidation theory of ENGLER (5) and BACH (1), wherein unsaturated bodies act as the carriers of oxygen, through intermediate peroxide formation. Might not a system made up of peroxide, peroxidase, and free oxygen, apparently simulating the condition at the flowering period, be able to oxidize a secondary alcohol to a ketone? It was assumed that the peroxide  , acting under the influence of peroxidase, might oxidize menthol to menthone in the following sense:





Of about twenty experiments carried out with this idea in mind, in not a single instance was oxidation found to take place.

While all the attempts to oxidize menthol by this system were fruitless, the results are reported because of the frequency in which such systems are resorted to in explanation of biological oxidations. The plant under consideration is an ideal one to test the validity of the hypothesis of ENGLER and BACH, for here are found in relative abundance the primary unsaturated compounds, which are known to be readily capable of peroxide formation, and thus bring about oxidations, according to the theories mentioned. Unfortunately such has not been found to be the case. KOSTYTSCHEW (8) has furnished an excellent review of the subject.

Experimentation

The technique was relatively simple and readily carried out. Three freshly prepared samples of α-pinene, β-pinene, and d-limonene were saturated with wet and dry oxygen, that is, oxygen was passed in until a small sample instantly decolorized an indigo solution of a color equal to that of a saturated solution of copper sulphate. In those protocols where the wet enzyme preparation was used, no further attempt to remove water other than drying on a force filter was made; while for those experiments where dry oxygen

was passed into the system, they were dried as far as possible on the force filter, and then in a vacuum desiccator over sulphuric acid. The enzyme preparation was not further purified than described later (9).

The extracts were obtained from sturdy plants at the height of the flowering period from the plat mentioned in a previous report (6). Twenty gm. of the fresh leaves was used as the source of the enzyme in most of the experiments. As soon as the plant material reached the laboratory, the leaves were stripped from the stems, shredded with scissors, ground with purified sea sand, and extracted with 200 cc. of water and allowed to digest for two hours. The filtered extract was treated with an equal volume of alcohol, filtered, and again taken up in 100 cc. of water and precipitated with alcohol.

All of the oxidase preparations yielded well defined reactions with the usual color reagents, including hydrothymoquinone.

EXPERIMENT I.—(a) 25 cc. α-pinene saturated with wet oxygen, 2 gm. menthol; (b) same as (a) but β-pinene used; (c) same as (a) but d-limonene used. The mixtures of menthol and oxygenated hydrocarbons were placed in 500 cc. round-bottom flasks, and set in direct sunlight for thirty days. The hydrocarbons were removed by distilling in vacuum and the residue tested for menthone by means of semicarbazide hydrochloride in the usual way. In all three cases a negative test was obtained, and the menthol was recovered almost quantitatively by steam distillation.

EXPERIMENT II.—The same result was obtained with hydrocarbons saturated with wet oxygen.

EXPERIMENT III.—Experiments I and II were repeated, but oxygen was passed into the mixtures for approximately eight hours a day for thirty days. No oxidation took place and the menthol was again recovered quantitatively.

EXPERIMENT IV.—A sample of oxidase obtained from 50 gm. of leaves and 2 gm. of menthol was allowed to stand at room temperature (about 23°) for fifteen days. A menthone test was negative and the menthol was recovered.

EXPERIMENT V.—Experiments I, II, and III were repeated but with the addition of an oxidase preparation from 20 gm. of leaves. The results were the same as before.

EXPERIMENT VI.—The test was carried out as for Experiment V, but oxygen passed in. The results were negative for menthone in all six cases, the menthol being almost totally recovered.

EXPERIMENT VII.—The protocol was carried out as the previous experiment, but shaken continuously for fifteen days. Identical results as previously mentioned were obtained.

EXPERIMENT VIII.—The previous experiments were modified by using ferrous sulphate as an additional catalyst, since it has been shown that ferrous salts acting as a catalyst on hydrogen peroxide may effect certain oxidations (4). The experimental mixture was made up as follows:

25 cc. α-pinene saturated with wet oxygen
2 gm. menthol
oxidase obtained from 20 gm. leaves
0.5 gm. of ferrous sulphate in 10 cc. of water.

When shaken a stable emulsion formed. The reaction flask was shaken continuously for two weeks and oxygen passed into the mixture for the duration of the experiment. The terpene components were recovered in the usual way and tested for menthone. A negative result was obtained.

EXPERIMENT IX.—The mixture was made up of 25 cc. of α-pinene saturated with oxygen, 2 gm. of menthol, and oxidase from 20 gm. leaves in 100 cc. of water. Oxygen was passed in for fifteen days and continuously shaken. The reaction mixture was worked up in the usual way. A negative test for menthone was obtained and the menthol recovered quantitatively.

As a final check on the absence of menthone, the combined menthol fractions were tested for menthone with semicarbazide hydrochloride in the usual way. No menthone semicarbazone was obtained.

These results conclusively show that the oxidizing enzymes, isolated from *Mentha piperita* L., alone or in the presence of so-called terpene peroxides, apparently simulating conditions in the plant, do not possess the power of oxidizing menthol to menthone. While quantitative results show the presence of considerable amounts of oxidizing enzymes in the plant, they must be considered as not related to a direct oxidation of menthol. Several experiments have

been completed in this laboratory, which serve to show that the menthol may be produced from menthone by a modified Cannizzaro reaction. In this case a reduction instead of an oxidation is involved, and is in agreement with the newer theories of WIELAND. These results will form the subject of a separate report.[2]

LABORATORY OF PLANT CHEMISTRY
UNIVERSITY OF WISCONSIN

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BRIEFER ARTICLES

A CHLORINATION METHOD FOR MACERATING WOODY TISSUES

(WITH ONE FIGURE)

FIG. 1.—Photomicrograph of vessel segment of *Magnolia;* ×120.

During recent investigations into the chemical nature of the cell walls of plants,[1] thin transverse sections of wood were exposed to the action of chlorine water followed by hot sodium sulphite solution to dissolve out the lignin. By this treatment the middle lamella was removed, leaving a residue of disorganized cells. This general reaction has been employed by chemists for some time in the preparation of wood cellulose, but in so far as the writer is aware, has received scant attention among anatomists. The method seems well adapted to fiber macerations of woody tissues.

Several woods were macerated by this method, which is apparently capable of yielding material in which the structural details of the various cells are faithfully preserved. Fig. 1 is a photomicrograph of a vessel segment of *Magnolia* isolated by the chlorine-sodium sulphite reaction. Details of structure are likewise preserved with equal clearness in parenchymatous elements, and excellent mounts can be made which seem to surpass those obtainable with the more drastic maceration methods now in general use. It seems probable as well that this procedure will adapt itself to studies of phloem tissues, since the writer was able to obtain seive tubes in fairly good condition from a maceration of *Populus* bark.

The method is as follows: (1) split out material match-stick size; (2) boil to expel air; (3) immerse in strong chlorine water for 2 hours; (4) wash; (5) immerse in hot 3 per cent sodium sulphite solution 15 minutes; (6) wash; (7) repeat the process from no.

[1] The chemical nature of the middle lamella. Tech. Publ. 21. N.Y. State Col. Forestry, Syracuse. 1927.

3 until, upon mild shaking, sufficient material separates for the purpose in view. When the red color first obtained upon addition of the sodium sulphite fails to reappear in this reagent after several chlorinations, the wood should be completely delignified.

Acknowledgments are due Dr. H. P. BROWN for helpful suggestions in preparing the manuscript.—WILLIAM M. HARLOW, *Department of Wood Technology, N.Y. State College of Forestry, Syracuse, New York.*

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CURRENT LITERATURE

BOOK REVIEWS

Soil conditions and plant growth

This valuable book by RUSSELL[1] has maintained its leadership in the field of knowledge covered, for fifteen years. It has now reached its fifth edition, and is the best presentation of the influence of soil conditions on the development of plants in the English language. The book needs no description, since it is so widely known and read. There are some changes in chapter headings, and in order of presentation they may be noted. Chapter IV, formerly the Colloidal Properties of the Soil, is now Chemical and Physical Relationships of the Soil. Chapter VII has been made chapter VI in the new edition, and chapter VI becomes chapter VII with the title Biotic Conditions in the Soil. The final chapter is entitled Methods of Soil Examination instead of Soil Analysis and its Interpretation. Appendix II of the former edition has been incorporated into the text.

RUSSELL is doing a splendid service for all students of the soil and plants in keeping this book up to date. The advances have been more rapid during the last six years than at any time in history, and every chapter has been amended to present the newer aspects of the soil relations. In commenting on the rapid advances made recently in the chemical investigation of the soil, as compared with the inactivity of this subject a few years ago, the author makes a statement that should not be forgotten by those who control the purse strings for research activity. He says: "Once again it has been shown that progress in applied science is limited by the state of knowledge of pure science, and that the surest way of solving technical problems is to investigate the underlying principles and causes." The book deserves a place in every library. The only criticism that one feels like making is that a book to be used as much as this one will be deserves to have a more durable binding.—C. A. SHULL.

Colorado plant life

The movement toward the popularization of science has resulted in the production of a series of non-technical books of the vegetation of certain limited areas. Among the better examples of this type of volume is RAMALEY'S description of the vegetation of Colorado.[2] This is not a local flora, it is much more, as may be seen from the following selection from its chapter headings: Plant

[1] RUSSELL, SIR E. J., Soil conditions and plant growth. 5th ed. 8vo. pp. viii+516. London: Longmans Green and Co. 1927.

[2] RAMALEY, FRANCIS, Colorado plant life. pp. 299. *figs. 133.* 3 colored plates. University of Colorado, Boulder. 1927. $2.00.

sociology; Life zones and altitude; Mountain parks; Mountain lakes; Mesas and foothills; Plants of the true mountains; Forests and forest trees; Flora of Colorado. In these chapters there is enough of the principles of plant ecology to interest and instruct the amateur naturalist, together with enough of actual contribution to knowledge to make the volume worth while for the professional botanist. For the former there are numerous illustrations, some of them well colored, and for the latter there are classifications of plant communities and critical notes on the associations of the various zones. The subalpine associations of the mountain parks, the stream, and the lake side have been carefully studied by the author, and the reader will profit from this rich experience.

The volume is one in a series of semi-centennial publications of the University of Colorado, and is well suited to extend the influence of the university far beyond its walls.—G. D. FULLER.

NOTES FOR STUDENTS

Origin and development of plant sociology.—In 1922 PAVILLARD, in an article entitled *Cinque ans de phytosociologie*, and previously noted in this journal,[3] ascribed the first recognition of this phase of plant science to HARPER.[4] This is perhaps correct, in so far as America and western Europe are concerned, but in eastern Europe it seems to have developed much earlier, and to have been recognized by PACZOSKI in 1896, who used the term in a sense practically identical with that accepted today. Later PACZOSKI[5] points out the heterogeneous structure of plant communities, which are built up of different species, while human society is relatively homogeneous. He stresses the influence of the plant population by pointing out that in spite of changes in environmental conditions, such as the draining of swamps, certain plant communities frequently persist for a long time, but changes come quickly as the result of the introduction of new elements in the plant population. He also states: "Phytosociology, as I conceive it, has to do with plant communities in a manner different from any other department of botany. The limits of the distribution of plants are rarely set by climatic factors. Often a plant does not grow in a given region because it is not able to compete with indigenous plants. This is so regardless of the fact that the climate is not unfavorable for it if it were growing alone (without competition). Phytosociology is the basis on which phytogeography has to be built, if the latter is to be a real science."

Almost simultaneously, according to ALECHIN,[6] the Russian scientists ac-

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[3] BOT. GAZ. 76:110–112. 1923.

[4] HARPER, R. M., The new science of plant sociology. Sci. Monthly 4:456–460. 1917.

[5] PACZOSKI, JOSEPH, Einige historische Angaben aus dem Gebiet der Phytosoziologie. Bot. Notiser 1925:320–324. 1925.

[6] ALECHIN, W. W., Wann und wo ist die Phytosoziologie enstanden? Bot. Notiser 1924:189–194. 1924.

cepted this phase of plant science, and KRYLOFF of the University of Tomsk, in 1898, wrote "The social relations of plants are being investigated. This branch of science is still young and may be called phytosociology." Further, in an introduction to a work on plant communities (Petrograd, 1915), SUKATSCHEW wrote "We must assume that phytosociology is now well established. The facts observed in a plant society resemble so closely those of human society that phytosociology best expresses this special branch of botanical science."

To these initial concepts may be added that of PAVILLARD and others, that phytosociology is the phase of plant science that endeavors to analyze vegetation inductively, by directing attention to the vegetation itself, rather than to the factors of the habitat. The most important unit is the association, and it aims to recognize and characterize all associations, designating them according to their characteristic or constant species. It has developed for itself a rather specialized terminology, as seen in the appearance at rather close intervals of two editions of a descriptive vocabulary.[7]

The two principal centers of its development have been termed respectively the Upsala and the Zurich-Montpellier Schools. Among the leaders of the former are DUERIETZ, GAMS, and ROMELL, while PAVILLARD, BRAUN-BLANQUET, and RÜBEL are prominent representatives of the latter.

In America sociological studies have not been very definitely separated from other phases of plant ecology. There have been, however, some investigations of plant populations, and among them three in which statistical methods have been applied to the vegetation of Michigan. GLEASON[8] has examined five virgin areas of maple-beech forest by means of strip count for the larger trees, and quadrat studies for the species of smaller size. The results of the census indicate a population of seventy-nine species, of which five trees, four shrubs, and fourteen herbs have a sufficiently high degree of frequency to be regarded as "characteristic species." *Acer saccharum* dominates the forest, with *Fagus grandifolia*, *Betula lutea*, *Ulmus americana*, and *Tilia americana* as important co-dominants. *Tsuga canadensis* is regarded as a relic of an earlier occupation of the land by coniferous forest. The deciduous forest is thought to be still young historically, and to have attained its full ecological dominance for only some three centuries.

A more extensive study of this association, with data on the percentage frequencies of the tree species in different portions of southern Michigan, has been made by QUICK.[9] Differences in the secondary species in different portions of the area are emphasized, and much importance is attached to historical factors

[7] BRAUN-BLANQUET, J., and PAVILLARD, J., Vocabulaire de sociologie végétale. 2d ed. p. 22. Montpellier. 1925.

[8] GLEASON, H. A., The structure of the maple beech association in northern Michigan. Papers Mich. Acad. Sci. Arts & Letters 4:285–296. 1924.

[9] QUICK, B. E., A comparative study of the distribution of the climax association in southern Michigan. Papers Mich. Acad. Sci. Arts & Letters 3:211–243. 1924.

and to competition. It is concluded that the beech-maple climax is capable, if left undisturbed, of occupying eventually all of the soils of the lower peninsula of Michigan, and of perpetuating itself on them.

KENOYER[10] has applied the methods of JACCARD and RAUNKIAER to a large number of plant associations in southwestern Michigan, determining the percentage of frequency occurrence for the individual species. More than 1000 determinations were made, and the results agree with those of RAUNKIAER in showing that species with lower frequencies are most numerous. When the species were arranged in five classes, on the basis of their frequencies, ranging from the least to the most abundant, the relative percentages of the number of species in the different classes were 70, 12, 6, 4, 7; in other words, the number of species in the least abundant class was ten times that in the most abundant. It was also found that when the number of species is small they tend to be confined to the first and the fifth groups; the same relationship was found to hold for the distribution of insects in grassland and for the distribution of microorganisms in hay infusion; and the method seems excellent for determining whether or not an association is uniform.

Similar results were obtained by KOOPER,[11] in a study of the weed population in the fall on rice fields of Java. Since these weed communities agreed with natural vegetation in respect to the relative numbers of constant and infrequent species, RAUNKIAER's law of distribution of frequencies seems to be fairly universal in its application. This investigation by KOOPER makes use of weed communities as indicators of the suitability of the different soils of the Pasuruan plain for the growth of different varieties of sugar cane.

Still more recently, FREY[12] has reviewed this statistical method with particular emphasis on the graphic expression of the results of such studies. Since JACCARD was the first to emphasize the fact that the number of species of the most frequent plants was less than that of the less frequent, the curves representing these relationships are termed by FREY "JACCARD's curves." Their use by RAUNKIAER, DURIETZ, and ROMELL is discussed, and several new possibilities of graphic representation are suggested. These are shown to be well suited to present such data as are now being published in investigations such as those conducted by the Zurich-Montpellier school of phytosociology. The application of GALTON's curve to the study of the association, by means of a "coefficient of significant species," seems to provide an exact method of testing the homogeneity of the plant population.

It is useless to attempt to enumerate the contributions of importance in

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[10] KENOYER, L. A., A study of Raunkiaer's law of frequence. Ecology 8:341–349. 1927.

[11] KOOPER, W. J. C., Sociological and ecological studies on the tropical weed vegetation of Pasuruan (island of Java). Rec. Trav. Bot. Neerlandais 24:x+252. 1927.

[12] FREY, ALB., Le graphique dans la phytosociologie. Rev. Gen. Bot. 39:533–546; 603–618. 1927.

this field, however, but a good list of the more important may be found in PAVILLARD'S recent review of the situation.[13] In this attempt to evaluate the progress that has been made, he points out the limitations of such statistical and mathematical methods as the formula of ARRHENIUS, and agrees with GLEASON[14] and others that the distribution of species within an association follows the recognized laws of chance and probability. He further advocates the importance of plasticity and open mindedness on the part of investigators, and warns them against being tied down to formulae or to a special terminology. He commends a recent article by COOPER[15] which emphasizes the universality of change in vegetation.

COOPER aptly compares vegetation to a stream: "The vegetation of the earth is presented as a flowing braided stream. Its constituent elements branch and interweave, disappear, and reappear. Vegetation as we see it today is the advancing front of this stream, in which we discern more or less definite groupings that tend to repeat themselves in space, the advancing termini of the streamlet elements. There is a constant tendency toward merging of related elements and thus toward simplification into fewer and larger currents, which is balanced by a trend toward multiplication of the streamlet elements through forking. The vegetational stream is governed and directed by the interaction of factors residing in the constituent organisms and in their environment."—G. D. FULLER.

Tertiary flora of Oregon.—CHANEY[16] has published an important report of his investigation of the Tertiary flora of a region of eastern Oregon. After describing the general geologic features, he describes the known flora of three localities. Major attention is given to the fossil flora of Bridge Creek. The material consists mostly of well preserved leaf impressions. The presentation of the Bridge Creek flora includes descriptions of 44 species in 39 genera, representing 28 families. Most of the species are woody dicotyledons, mainly arborescent, only one fern, four conifers, and two monocotyledons representing these groups. Ten new species are described, and there is a full discussion of all the species. This region is especially favorable for an investigation of the ancient flora, since the material occurs in volcanic shales, which split readily along the bedding planes, making available an abundance of usable material.—J. M. C.

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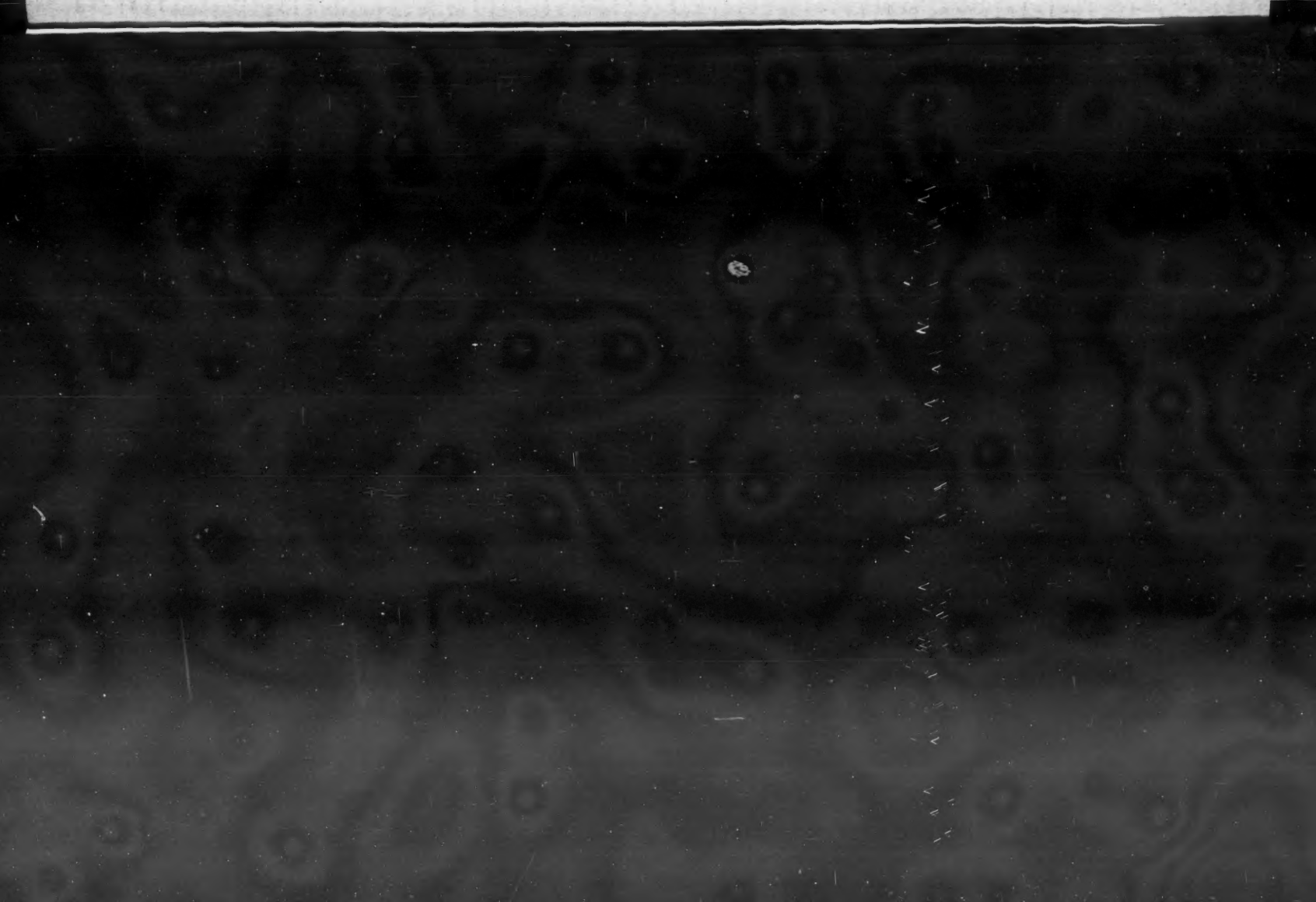

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